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THE USE OF Δ^5-PREGNENOLONE IN VARIOUS CLINICAL DISORDERS*

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THE clinical effectiveness of cortisone and pituitary adrenocorticotropic hormone (ACTH) in rheumatoid arthritis and allied collagen diseases has stimulated the investigation of more easily derived steroids in these conditions. The possibility exists that certain steroids, even though not themselves hormonally active, may be converted in the human organism to materials which are useful in the treatment of the aforementioned diseases. With this in mind we have administered the steroid, Δ^5-pregnenolone (17-ethyl-Δ^5-androstene-3β-ol-20-one), to a group of 59 patients

Δ^5-Pregnenolone, hereinafter simply designated pregnenolone, is the 3-hydroxy analogue of progesterone, and can be obtained during the oxidative preparation of the latter hormone (1, 2, 3). It was first prepared chemically by Butenandt (4) in 1934, and was obtained by extraction from hog testes in 1943 by Ruzicka (5), and in 1948 by Kuizenga (6). Despite its relatively early synthesis, data regarding its action have accumulated slowly. They have been well summarized in the review of Henderson, Weinberg and Wright (7).

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In rats, no toxic effects have been observed following the use of large doses of pregnenolone by oral, subcutaneous or intraperitoneal routes of administration (8). The steroid appears to be equally harmless to man (9–17), except for the occasional development of an allergic reaction or an abscess at the site of injection (13).

Early tests with pregnenolone for estrogenic, progestational, adrenocortical and gonadotropic effects revealed questionable activity in the doses used (12, 18, 20–23). However, with much larger amounts it is possible to maintain spermatogenesis and the weight of the testes in hypophysectomized rats (24–26). The first physiologic activity in man was reported by Hoagland in 1944 (27) who found that pregnenolone, administered orally, reduced the urinary excretion of 17-ketosteroids during fatigue. Davison and Koets (10, 11) observed an increase in the excretion of 17-ketosteroids in ankylosing spondylarthritis, and used pregnenolone in this condition with the thought that it might reduce the rate of ketosteroid excretion. This did occur in conjunction with marked clinical improvement. Recent reports (7, 12, 13, 17, 28–30) indicate that pregnenolone is clinically effective in the treatment of rheumatoid arthritis.

SUBJECTS AND METHODS

The individuals selected for this study included 30 patients with various types of arthritis: 11 with the rheumatoid form, 13 with hypertrophic osteo-arthritis, 3 with gouty changes, 1 with acute arthritis of undetermined etiology, 1 with allergic hydrarthrosis and 1 with Still's disease. Four patients had chronic lupus erythematosus; 3, psoriasis; 2, acute disseminated lupus erythematosus; 2, scleroderma; 7, Addison's disease; 4, malignant exophthalmos; and 1 each, Hodgkin's disease, tuberculosis of the spine, obesity, abdominal pain due to chronic pancreatitis, erythema nodosum, chorioretinitis, amyotrophic lateral sclerosis and chronic cystic mastitis.

Of the 59 patients treated with pregnenolone, 24 were males and 35 females. Their ages ranged from 11 to 86 years. Twenty-three of these patients were hospitalized for at least a portion of their period of treatment.

In conjunction with the management of each individual patient certain laboratory procedures were routinely carried out prior to therapy, and repeated when indicated at one-week to two-week intervals during treatment, namely: urinalyses; blood counts; sedimentation rates; sugar tolerance tests; basal metabolic rates; blood nonprotein nitrogen, urea nitrogen, and glucose; roentgenograms; electrocardiograms; and biopsies. Eosinophil counts and uric acid-creatinine ratios following the administration of epinephrine and ACTH were performed in some of the subjects

according to the methods of Thorn and coworkers (31) with the modifications suggested by Randolph (32).

Fifteen patients who were under continuous supervision in the hospital were given special diets for specified periods of time, before, during and after which their daily balances for nitrogen and electrolytes were determined. The details of this work are recorded elsewhere (33).

All patients were given pregnenolone in aqueous suspension[1] by daily intramuscular injection. Late in the course of therapy, some of the subjects received acetoxy-pregnenolone or pregnenolone acetate by mouth, but results of this supplementary therapy are not of primary concern here. The period of therapy varied from four to one hundred and sixty-seven days. The average course of treatment was approximately forty days. The daily dose of pregnenolone varied from 50 to 600 mg., with an average of about 250 mg.

Clinical observations were recorded daily. Subjective symptoms, such as a sense of well being,[2] changes in skin texture and elasticity, alteration of pain and joint motility, and variations in eye signs, were noted. For instance, in each of the arthritic group of patients particular attention was paid to variations in rubor, temperature and swelling about the joints, the degree of mobility, fluctuations in body temperature and sedimentation rate.

RESULTS

The results can best be considered by dividing the patients into 3 groups: a) those with arthritis of various types, b) those with "collagen diseases," and c) those with endocrine dysfunctions and miscellaneous disturbances.

A. Arthritis

In this group there were 11 patients with rheumatoid arthritis. Five of these patients were male, and 6 were female. Their ages ranged from 34 to 65 years. All of these were in an active stage of the disease (Tables 1 and 4) according to the classification of the N.Y. Rheumatism Association (34). Six of these patients showed moderate to marked improvement, and 5 improved slightly or not at all. The first evidence that therapy was effective occurred between the second and fourth days after the beginning of treatment. This subjective sensation of well being was usually associated with an increased appetite. Between the fourth and eighth days there was a definite diminution in the degree of joint pain with, not infrequently,

[1] Generous supplies of this material were made available through the courtesy of Dr. Edward L. Henderson, Medical Director, Schering Corporation.

[2] Sense of well being, used to indicate an euphoric state with increased strength and vigor.

TABLE 1. EFFECT OF PREGNENOLONE ON 30 PATIENTS WITH VARIOUS TYPES OF ARTHRITIS

Case	Age (yrs.)	Sex	Diagnosis	Duration of disease	Stage* & location**	Class* (severity)	Total dose (Gm.)	Total duration of treatment (days)	Avge. daily dose (mg.)	Improvement***	Remarks
1	65	F	R.A.†	3 yrs.	IV (p.j.)	IV	30.20	123	245	++	Increased motility; diminished pain; sense of well-being.
2	53	M	R.A.	7 mos.	III (p.j., s.)	III	30.85	97	318	+++	Pain much diminished; increased joint motility
3	49	F	R.A.	13 yrs.	III (p.j.)	IV	9.20	23	400	+++	Ambulatory in 1 wk.; joint motility increased; pain diminished.
4	64	M	R.A.	2 mos.	II (p.j.)	IV	4.70	28	168	+++	Joint pain and swelling markedly improved.
5	40	M	R.A.	2½ yrs.	IV (p.j., s.)	III	9.30	51	182	+	Sense of well being; slight increase in joint motility.
6	42	F	R.A.	3 yrs.	II (p.j.)	III	5.30	28	189	+	Slight diminution of pain and increased motion.
7	49	F	R.A.	3–5 yrs.	IV (s.)	II	4.40	22	200	+	Slight diminution of pain and stiffness, first 7 days.
8	63	F	R.A.	2 yrs.	III (p.j.)	I	5.40	18	300	+++	Improvement began after 2nd day; diminished pain; well-being.
9	75	M	H.O.A.‡	2 yrs.	IV (b.h.)	II	1.60	8	200	0	Injections too painful to continue.
10	69	F	H.O.A.	20 yrs.	III (k.)	II	7.60	48	158	++	Sense of well being; pain diminished; stiffness the same.
11	75	F	H.O.A.	1 yr.	II (k.)	IV	0.95	16	59	+++	Marked diminution of pain; ambulatory in 1 wk.
12	53	F	H.O.A.	3 wks.	II (k.)	IV	1.70	17	100	+++	Marked improvement in pain and motility.
13	58	F	H.O.A.	3 yrs.	II (k.)	III	3.80	21	181	+	Slight diminution of pain.
14	58	F	H.O.A.	2 yrs.	III (p.j.)	IV	2.77	30	92	+	Slight improvement in walking after 7 days.
15	55	M	H.O.A.	1 yr.	II (sh.h.)	II	3.90	21	185	0	No improvement.
16	54	M	Gout	1 mo.	I (h.)	I	1.30	8	162	+	Slight sense of well-being; diminution of pain.
17	53	M	Gout	6 mos.	II (t.a.)	II	2.77	30	92	+++	Marked diminution of pain; tophi cleared.

TABLE 1. EFFECT OF PREGNENOLONE ON 30 PATIENTS WITH VARIOUS TYPES OF ARTHRITIS

Case	Age (yrs.)	Sex	Diagnosis	Duration of disease	Stage* & location**	Class* (severity)	Total dose (Gm.)	Total duration of treatment (days)	Avge. daily dose (mg.)	Improvement***	Remarks
18	46	F	A., ac.§	2 wks.	II (p.j.)	III	2.25	27	83	+++	Swelling and pain markedly diminished.
19	38	M	Allergic hydrarthrosis	6 yrs.	II (k.h.)	I	14.60	28	521	0	
20	11	F	Still's disease	6½ yrs.	IV (p.j., s.)	IV	8.50	55	154	+++	Sense of well-being; pain markedly diminished by 4th day.
21	63	F	H.O.A.	3 yrs.	III (p.j., k.)	III	6.30	21	300	++	Striking relief of pain.
22	66	M	H.O.A.	8 yrs.	III (p.j., h.sh.k.)	II	18.30	52	350	+++	X-ray appearance of hands is that of R.A.
23	86	M	H.O.A.	20–25 yrs.	III (s.)	II	5.10	17	300	+++	
24	67	F	H.O.A.	20–30 yrs.	III (s.sh., hips, h.k.)	II	14.00	40	350	+++	
25	51	F	H.O.A.	5 yrs.	III s.h., k.)	II	14.00	35	400	0	
26	50	M	Gout	10 yrs.	Metatarso-Phal.-L. big toe	I	8.00	60	400	+	
27	52	F	H.O.A.	1 yr.	II (p.j., s.h.k. wrist)	II	8.40	28	300	+++	Obese; high blood pressure; sed. rate 3/42.
28	34	M	Marie-Strûmpell (R.A.)	6 yrs.	III (s.)	II	3.10	21	200–500	+	Also had iritis & multiple allergies.
29	58	M	Marie-Strûmpell (R.A.)	27 yrs.	III (s.h.: wrists, k.)	III	5.40 (i.m.)	22	135	+	Treated every 2–4 days; multiple allergies.
							10.00 (oral)	35	300	+	
30	46	F	R.A.	5 yrs.	I (s.h.)	II	3.60 (i.m.) 9.20 (oral)	144	75 (i.m.) 400 (oral)	+++	Estrogen did not help.

* Stage and class in accordance with therapeutic criteria of N.Y. Rheum. Association.
** p.j.—peripheral joints, s.—spine, b.—back, h.—hands, k.—knees, sh.—shoulders, t.—toes, a—ankles.
*** Improvement graded as follows: 0 —no improvement, + —slight improvement, ++ —moderate improvement, +++ —marked improvement.
† R.A. is rheumatoid arthritis.
‡ H.O.A. is hypertrophic osteoarthritis.
§ A., ac. is acute arthritis.

a slight increase in joint mobility. Patients 2, 4 and 8 (Table 1), in whom joint pain due to rheumatoid arthritis was severe, had marked relief of pain in from two to seven days after pregnenolone was started. Patients 3 and 4 (Table 1) showed a diminution in the swelling of the peripheral joints after the first week of therapy. A progressively favorable influence continued until about the fourth week of therapy, following which little further regression in the disease process could be produced by the continuation of pregnenolone, even though the dosage was increased. As a rule, however, the degree of improvement achieved by the fourth week could be maintained with continued therapy. In responding patients, partial ambulation was usually possible during the second week. The improvement in appetite and the sense of well being usually continued during the entire period of treatment. A slight increase in weight was common. During the first week a small but appreciable decrease in sedimentation rate frequently occurred, but in a majority of instances this returned subsequently to previous levels despite clinical improvement. Body temperature was only slightly affected, although the trend was toward normal. There was no definite effect of pregnenolone upon the eosinophil count and the white blood cell count (Figs. 1 and 2). The rate of excretion of 17-ketosteroids in the urine showed a fall during the first few days of treatment, followed by a return to previous levels. There was occasional recurrence of joint pain during the period of treatment, but this was usually transient. When therapy was stopped there was a definite tendency for all symptoms to recur within a few days. Re-establishment of therapy with pregnenolone promptly resulted in clinical improvement.

There were 13 patients with hypertrophic osteoarthritis who were treated with pregnenolone. Nine of these patients were female, 4 were male, and their ages ranged from 51 to 86 years. Three of these subjects were hospitalized, and 10 were treated in a similar manner while ambulatory. Seven of these patients showed a moderate to marked improvement as a result of treatment with pregnenolone, and 6 obtained little or no relief. A sense of well being with increased appetite was commonly experienced within two to four days after beginning treatment. Within five to eight days pain decreased and range of motion about the joints was increased. Amelioration of joint pain was prominent in Patients 11, 12, 22, 24, and 30 (Table 1). Patients who were bedridden usually became ambulatory in ten to fourteen days. Improvement in ability to climb stairs was a simple but reliable indication of a favorable response to the drug. After four to five weeks of gradual improvement, the symptoms of the disease could not be further influenced, regardless of the dose of pregnenolone employed. On cessation of therapy, joint pains usually recurred. Temperature,

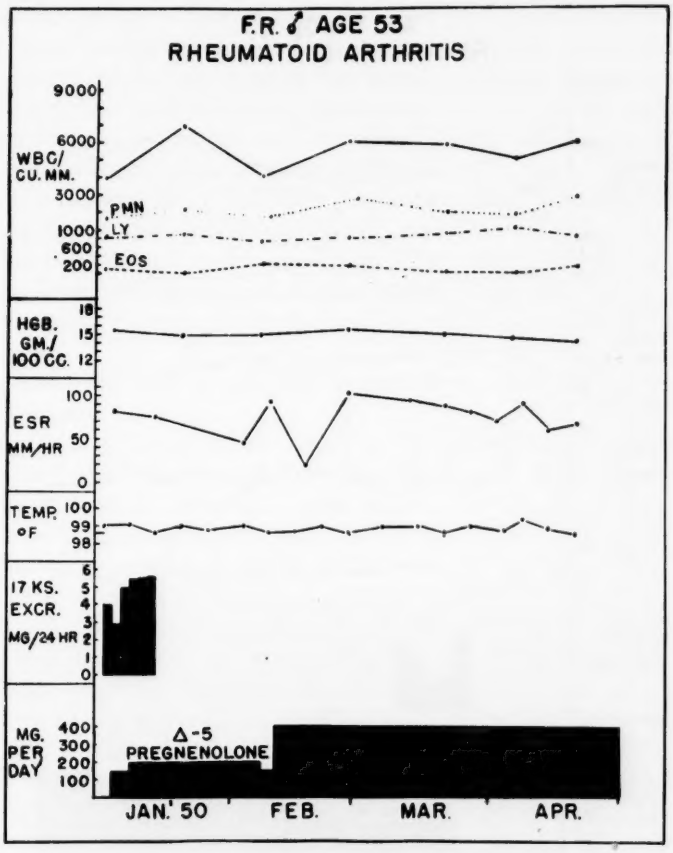

FIG. 1. Fluctuations in white counts, hemoglobin, sedimentation rate, body temperature and 24-hour excretion of 17-ketosteroids during therapy with pregnenolone in a patient with rheumatoid arthritis (Case 2).

white blood cell counts, eosinophil counts and sedimentation rates were unaffected by therapy with pregnenolone. A majority of the patients gained a slight amount in weight during the period of treatment, usually without relation to the degree of amelioration of pain in the joints.

Of the 3 patients with gouty arthritis who were treated with pregnenolone, 1 obtained a dramatic response. He had previously received various forms of medication, including colchicine, but without relief. Within three days after beginning pregnenolone the joint pains subsided, and the patient was able to resume his usual occupation. A sense of well-being was present during the entire period of treatment. After ten days of therapy

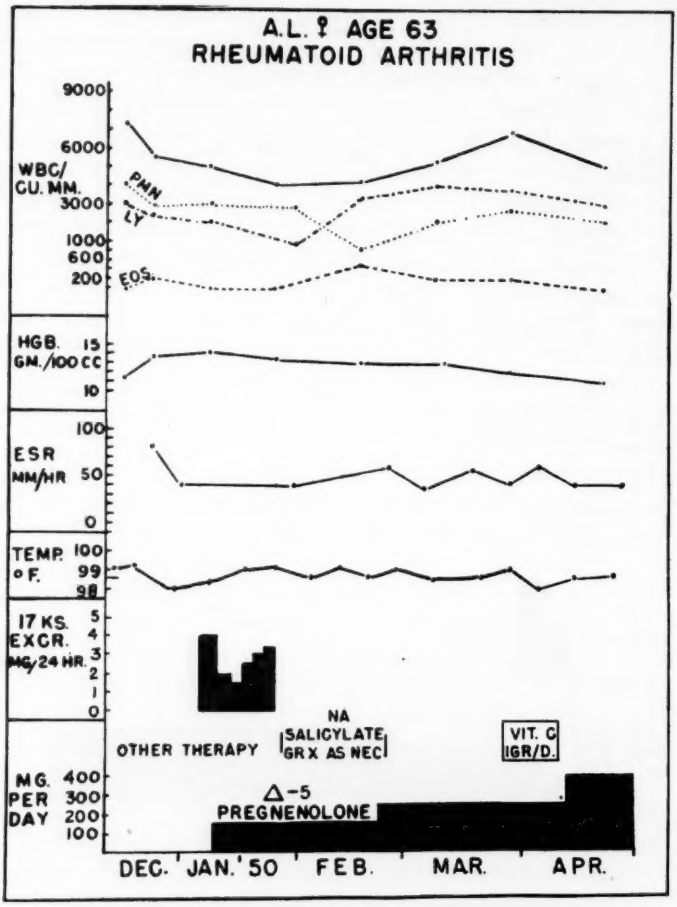

FIG. 2. Fluctuations in white count, hemoglobin, sedimentation rate, body temperature and 24-hour excretion of 17-ketosteroids during therapy with pregnenolone in a patient with rheumatoid arthritis (Case 8).

it was noted that the tophi on the ears were becoming smaller. Blood uric acid levels were uninfluenced by the drug. Following four weeks of treatment with 300 mg. of pregnenolone daily, this patient was given a weekly injection of 200 mg. of pregnenolone with only an occasional brief return of symptoms. The other 2 patients with gouty arthritis were benefited slightly by the pregnenolone, but 1 of these received treatment for only eight days. In this instance the drug was discontinued because of the development of a red indurated area at the site of injection.

A 46-year-old white female who developed an acute polyarticular arthri-

tis of undetermined etiology was treated for a period of twenty-seven days with pregnenolone. Marked recession of pain and swelling of the joints occurred during the first five days of therapy. In association with treatment, the hemoglobin rose from 10.0 to 11.5 Gm. per 100 cc., body weight increased slightly and fever disappeared, but the leucocyte count and the sedimentation rate were unaffected.

An 11-year-old girl with Still's disease of six and a half years' duration received pregnenolone for a period of one hundred and fifty-four days. Within four days after starting therapy the patient noted a marked sense of well-being with diminution of joint pains. In this case the sedimentation rate showed a decided fall in conjunction with the administration of pregnenolone.

One patient with an allergic hydrarthrosis received no benefit from pregnenolone.

The over-all picture of the treatment of various types of arthritis with pregnenolone (Table 4) indicates that the drug is effective in about half the cases, with a somewhat higher incidence of improvement in the patients with rheumatoid arthritis. Of 14 patients with various types of arthritis, the severity of which could be graded as III or IV (34), 8 had moderate to marked relief of symptoms for periods of four weeks to six months during therapy. In 18, or 60 per cent of our patients with arthritis, there was a return of symptoms in two to five weeks after discontinuing the drug. Some of the remainder are still being treated. Five have been given cortisone with further improvement. Seven have been watched for from two to seven months after therapy was stopped, without any observed relapse. It appears, therefore, that pregnenolone influences various types of joint pain, seemingly regardless of the nature of underlying etiologic factors or pathologic changes.

B. Lupus erythematosus

Of 11 patients with conditions classified as collagen diseases, who received therapy with pregnenolone (Tables 2 and 4), 4 suffered from chronic lupus erythematosus, involving principally the face. All were women, whose ages ranged from 24 to 51 years. They had had the skin lesions intermittently for from six to twenty years with very little effect derived from a wide variety of therapeutic measures. Under treatment with pregnenolone, 3 of the 4 patients showed a moderate to marked improvement. As a rule, after two or three days of therapy there was a sense of well-being. The first noticeable effect upon the skin lesion was apparent about the third day. This was evidenced by a definite diminution in the degree of redness (a blanching effect) with a recession at the periphery of the involved area. In Patient 37 (Table 2) there was almost complete disappear-

TABLE 2. EFFECT OF PREGNENOLONE ON 11 PATIENTS WITH COLLAGEN DISEASES

Case	Age (yrs.)	Sex	Diagnosis	Duration of disease	Severity*	Total dose (Gm.)	Total duration of treatment (days)	Avge. daily dose (mg.)	Improvement**	Remarks
31	53	M	Ps.†	3 yrs.	I	30.85	97	318	+	Slight clearing of lesions.
32	40	M	Ps.	1 yr.	II	9.30	51	182	+++	Scalp lesions cleared in few days.
33	41	M	Ps.	Many yrs.		6.00	20	300	±	Very questionable change.
34	65	F	Scl.‡	1 yr.	III	30.20	123	245	+++	Marked softening of skin by end of 2nd wk.; able to wrinkle brow.
35	56	F	Scl.	6 mos.	II	6.00	15	400	+	Skin softened over forearms & around fingers by end of 1st wk.
36	51	F	L.E., Ch.#	15 yrs.	III	18.40	46	400	0	Questionable reduction in redness.
37	39	F	L.E., Ch.	1½ yrs.	II	5.60	27	200	+++	Almost complete clearing of skin lesions.
38	32	F	L.E., Ch.	6 yrs.	II	6.00	15	400	++	Less redness; lesions less moist.
39	24	F	L.E., Ch.	7 yrs.	IV	7.95	81	98	+++	Rash almost disappeared; joint pains diminished.
40	24	F	L.E., Ac.§	3 mos.	IV	2.40	4	600	0	Too short a period of treatment.
41	34	F	L.E., Ac.	3 mos.		1.20	2	600	0	Going downhill; switched to cortisone (30 mg.); died but dose of cortisone not high.

* Severity graded as Class I, II, III, IV—slight, moderate, marked, very severe.
** Improvement graded as follows; 0 —no improvement; + —slight improvement; ++ —moderate improvement; +++ —marked improvement.
† Ps. is psoriasis.
‡ Scl. is scleroderma.
# L.E., Ch. is chronic lupus erythematosus.
§ L.E., Ac. is acute disseminated lupus erythematosus.

ance of the rash from the face within fourteen days after treatment was begun. When the drug was stopped and hypodermic injections of sterile saline were substituted, the rash recurred. It faded again when therapy with pregnenolone was re-instituted. Case 39 (Table 2) was that of a 24-year-old girl with a seven-year history of a skin rash and joint pains. All previously used forms of therapy had failed. After the third day of treatment with pregnenolone the joint pains subsided, the appetite improved and the dermatologic lesion began to recede. Three weeks later the sedimentation rate was 80 mm. in one hour as compared with 160 mm. prior to treatment. This patient has remained on continuous daily injections of 100 mg. of pregnenolone because all symptoms recur when the drug is discontinued. The only patient with chronic lupus erythematosus who did not respond to pregnenolone was a 51-year-old woman with a discoid type of lesion over the chin, malar eminences and chest wall. Considerable scarring was present in the lesions. It seems likely that pregnenolone will not influence lesions which extend quite deeply into the subcutaneous tissues.

Two women with acute disseminated lupus erythematosus were treated for two and four days respectively with daily injections of 600 mg. of pregnenolone. No response was noted in either.

C. Scleroderma

Two white women with scleroderma confirmed by skin biopsy, ages 56 and 65 years respectively, were treated with pregnenolone. By the end of the first week of treatment there was noted a definite softening of the skin over the face, forearms and hands. Secondary symptoms due to the tightening of the skin were lessened. Improvement in the texture and elasticity of the skin continued for about the first five weeks of therapy, after which no further progress in the softening process occurred; but that which had been achieved was maintained.

D. Psoriasis

Three patients, all men, aged 40, 41 and 53 years, respectively, with psoriasis of one "many," and three years' duration, respectively, were treated with marked improvement in 1 case, and little or no change in the other 2 (Patients 31, 32 and 33, Table 2). Improvement in 1 of these with diffuse involvement of the scalp was marked within three days, and was characterized by diminution of the scaling and almost complete disappearance of lesions in four to six weeks. There has been no recurrence of lesions following cessation of drug therapy several weeks ago.

Twelve patients with various types of endocrine disorders were treated with pregnenolone (Tables 3 and 4).

TABLE 3. EFFECT OF PREGNENOLONE ON PATIENTS WITH ENDOCRINE AND MISCELLANEOUS DISORDERS

Case	Age (yrs.)	Sex	Diagnosis	Duration of disease	Symptoms	Total dose (Gm.)	Total duration of treatment (days)	Avge. daily dose (mg.)	Improvement*	Remarks
42	40	F	Mal. exoph.**	20 yrs.	Tearing; exoph.	9.60	24	400	+++	Lid closure improved; less tearing & less edema.
43	35	M	Mal. exoph.	1 yr.	Tearing; exoph.	22.00	55	400	++	Exoph. slightly improved; tearing, & edema markedly improved.
44	51	F	Mal. exoph.	15 yrs.	Tearing; conjunctivitis	2.45	26	94	+++	Exoph. slightly improved; tearing & edema markedly improved.
45	66	M	Mal. exoph.	9 mos.		6.80	21	400	+++	Exoph. (before, 22; after, 20); diplopia not affected.
46	26	F	Add.†			0.70	7	100	0	
47	58	F	Add.			11.50	167	68	+	Sense of well-being; lost weight with small doses.
48	28	F	Add.			5.95	119	50	0	
49	33	M	Add.			0.80	4	200	0	Injections too painful to continue.
50	53	M	Add.			2.10	7	300	0	Felt worse on pregnenolene.
51	34	F	Add.			0.40	4	100	0	
52	31	F	Chronic cystic mastitis	4 yrs.		7.30	41	300 (21 d.) 50 (20 d.)	+++	Breast lumps disappeared; less tired.
53	54	F	Obesity	8 yrs.	Edema extrems.	11.10	90	123	0	
54	46	F	Hodgkin's disease	4 yrs.	Pruritis	6.00	20	300	0	
55	58	M	Pancreatitis, ch.	10 yrs.	Abd. pain	3.50	21	167	0	
56	41	M	Tbc. spine	4 yrs.	Pain in spine	5.50	30	183	0	
57	45	M	Chorioretinitis	2 mos.	Dim. vision	5.60	14	400	+++	Moderate improvement in vision.
58	15	F	Erythema nodosum	3 mos.	Painful nodes	7.60	19	400	+	Slight fading of skin lesions.
59	49	M	Amyotrophic lateral sclerosis	12 yrs.		5.60	14	400	++	Appetite markedly improved; euphoric; nerve lesions unchanged.

* Improvement graded as follows: 0 —no improvement; + —slight improvement; ++ —moderate improvement; +++ —marked improvement.
** Malignant exophthalmos.
† Addison's disease.

TABLE 4. COMPOSITE CHART OF 59 PATIENTS TREATED WITH PREGNENOLONE

Diagnosis	No. of cases	Age range (yrs.)	Sex	Improvement		Percentage improvement	
				Unimp. & slight	Moderate & marked	Unimp. & slight	Moderate & marked
Rheumatoid arthritis	11	34–65	6 M 5 F	5	6	45.5	54.5
Hypertrophic osteoarthritis	13	51–86	4 M 9 F	6	7	46.2	53.8
Gouty arthritis	3	50–54	3 M	2	1	66.7	33.3
Acute arthritis	1	46	F	0	1		
Allergic hydrarthosis	1	38	M	1	0		
Still's disease	1	11	F	0	1		
Total	*30*	*11–86*	*14 M* *16 F*	*14*	*16*	*46.7*	*53.3*
Lupus erythematosus, chronic	4	24–51	4 F	1	3	25.0	75.0
Lupus erythematosus, acute	2	24–34	2 F	2	0		
Psoriasis	3	40–53	3 M	2	1	66.7	33.3
Scleroderma	2	56–65	2 F	0	2		
Malignant exophthalmos	4	35–66	2 M 2 F	0	4		100.0
Addison's disease	6	26–58	2 M 4 F	6	0	100.0	
Hodgkin's disease	1	46	F	1	0		
Obesity	1	54	F	1	0		
Pancreatitis, chronic	1	58	M	1	0		
Erythema nodosum	1	15	F	1	0		
Chorioretinitis	1	45	M	1	0		
Chronic cystic mastitis	1	31	F	0	1		
Amyotrophic lateral sclerosis	1	49	M	0	1		

E. Endocrine disorders

Four of these patients had malignant exophthalmos, the onset of which followed thyroidectomy. Their ages lay between 35 and 66 years. They showed remarkable changes under the influence of pregnenolone. A sense of well-being occurred within two or three days after therapy was started. The first objective evidence of a clinical change due to the drug was manifested on the third to fifth days of treatment, with diminution in the periorbital edema, and less difficulty in closing the eyes. Simultaneously, tearing and conjunctivitis were lessened. Exophthalmometric readings disclosed a slight diminution in Patients 42, 44 and 45 (Table 3), but no essential change in Patient 43. The subjective and objective improvement in the 4 cases was marked, despite the relatively small change in the exophthalmos.

Six patients with Addison's disease were given pregnenolone for periods of time ranging from four to one hundred and sixty-seven days. Four of these patients were women, and 2, men. Their ages ranged from 26 to 58 years. The clinical condition of 5 of these patients was unchanged by pregnenolone, and 1 individual showed slight improvement as manifested

by an increase in strength and vigor. Changes towards normal in the serum electrolytes and blood pressure readings were lacking in all of these patients. Thus, it appears that pregnenolone had no definite effect on the clinical status of patients with adrenal cortical insufficiency.

Pregnenolone was without effect in 1 patient with obesity.

In 1 subject, the lumps of "chronic cystic mastitis" could no longer be palpated following several weeks of treatment with pregnenolone.

F. Miscellaneous conditions

No clinical benefit was obtained in 1 patient each, who suffered from Hodgkin's disease, abdominal pain due to chronic pancreatitis, tuberculosis of the spine, and erythema nodosum. One patient with chorioretinitis of undetermined etiology was apparently helped by pregnenolone. However, since the patient's condition began to improve shortly before receiving the drug, no credit for its action has been claimed.

Euphoria and improved appetite were observed as a result of therapy with pregnenolone in 1 subject with amyotrophic lateral sclerosis (Table 3), but over a short period of time no change in the essential lesions of the disease were observed.

G. Toxic reactions to pregnenolone

No systemic reactions due to pregnenolone were encountered. Occasionally, redness, pain and induration developed at the site of an injection.

DISCUSSION

A. Indications for pregnenolone therapy

As a result of treatment with pregnenolone, we have found improvement in the clinical condition of patients suffering from rheumatoid arthritis, hypertrophic osteoarthritis, chronic lupus erythematosus, malignant exophthalmos and scleroderma. Others have reported a similarly good influence upon rheumatoid arthritis (7, 10, 12, 13, 17, 28, 29, 30, 35). The recent communication of Guest and his associates (36), in which poor results are described with pregnenolone in rheumatoid arthritis invites careful scrutiny. Both inadequate dosage and insufficient length of treatment appear to be factors in their unsatisfactory usage of pregnenolone. It has been our experience that, for adequate therapy, we must use a daily injection of 200 to 600 milligrams of the steroid or a corresponding amount of the orally administered acetate. It has been our practice to increase the daily dosage quickly during the first ten to fourteen days, using as much as 600 milligrams per day when necessary. If a beneficial action is not obtained within this period of time, using the largest doses of the

drug, then further attempts at treatment with pregnenolone should be abandoned. As a rule, an adequate course of treatment takes from three to five weeks. The maximum effect is generally obtained within this period of time, and further improvement will not occur.

B. Pregnenolone in arthritis

The behavior of pregnenolone in our cases of rheumatoid arthritis, hypertrophic osteoarthritis, Still's disease, acute arthritis, and gout leads us to the conclusion that benefit, when obtained, has shown some degree of relationship which is usually directly proportional to the amount of active inflammatory reaction. In other words, longstanding articular and periarticular lesions are less susceptible to a favorable influence than the lesions of recent and obviously inflammatory nature. For instance, in osteoarthritis the joint lesion itself is one of little or no inflammatory reaction. In these cases, most, if not all of the changes we have observed have occurred in the periarticular lesions, such as the fibrositis and myositis.

The pattern of response to pregnenolone was in its broad general outlines rather constant. The earliest clinical changes were usually noted between the third and the seventh days after therapy was started. A "sense of well-being" and an increased appetite were the first responses to pregnenolone. These were usually noted not later than the fourth day. These effects might often appear before there were any noticeable alterations in the joint pains or other clinical manifestations. With improvement in pain, joint mobility was increased, active motion encouraged, and muscular atrophy reduced. There was a definite tendency for joint manifestations to recur when therapy was stopped, but reinstitution of treatment again proved beneficial. Occasionally a flare-up of acute symptoms would occur in some joints during treatment with pregnenolone, but these symptoms proved to be transient in nature provided treatment with large doses of the drug was continued.

Despite the above evidences of clinical improvement, pregnenolone caused little or no change in the white count, the eosinophil count or the sedimentation rate. In 1 of our cases (Case 20, Table 1) the sedimentation rate fell dramatically. In general, however, there was a slight tendency for it to fall only in the early period of treatment followed, by a return to previous levels.

C. Nature of pregnenolone activity

Elsewhere we have described the tendency for pregnenolone to produce a negative balance for nitrogen and potassium, and a questionably positive balance for sodium and chloride (33). At this point we are more

concerned with the theoretical considerations arising from its use in the management of the diseases mentioned in the summary table (Table 4). The ability of pregnenolone to affect the tissues beneficially in chronic lupus erythematosus, scleroderma, psoriasis and malignant exophthalmos was in most instances striking. The common denominator of change in these conditions appeared to be concerned with changes in the capillaries and supporting matrix of the skin and mucous membranes. The mechanism of the action is obscure; several possibilities may be considered:

(i) *The action may be that of cortisone:* Several things make this unlikely. Cortisone produces improvement not only in the conditions mentioned (with the exception of malignant exophthalmos, for which, as far as we know, it has not been tried), but also in acute disseminated lupus erythematosus and Addison's disease, for which pregnenolone was of no help.

(ii) *The action maybe upon the pituitary*: Selye and Clarke have observed the potentiation of the action of certain pituitary extracts by the concomitant administration of pregnenolone (21). The relief of the collagen diseases might be ascribed to such an action, working synergistically with adrenocorticotropin (ACTH) by way of the adrenal. Although this would explain the lack of effects in Addison's disease, it would not explain the failure to influence acute lupus erythematosus favorably. Moreover, the favorable action upon malignant exophthalmos may indicate a suppressive effect upon the pituitary, at least insofar as its formation of thyrotropin is concerned.

(iii) *A functioning adrenal cortex may be essential to the action:* The practically complete failure of the steroid to influence the course of Addison's disease in 6 consecutive cases lends support to the supposition that the adrenal cortex must be functionally active. The action on the preputial glands of castrated female rats is diminished if the adrenals are absent (21). On the other hand, life of adrenalectomized animals seems to have been prolonged by the use of pregnenolone (22, 23), but many features of corticoid activity are lacking (37, 38).

Despite the close relationship between the chemical structure of pregnenolone and progesterone, no positive evidence that such a conversion or any other transformation takes place in the human body has been brought forward, although the possibility has been suggested (27) and such a transformation proven in regard to the isolated perfused adrenal gland (27A). It seems likely, therefore, that an intact adrenal will favor the action of pregnenolone by its conversion to a more active hormone, although the variety of its pharmacologic and hormonal actions lends weight to the concept that it may also exert a quite individualistic action of its own (7, 33).

(iv). *The salutory action of pregnenolone may be due to a direct effect upon the involved tissues:* The collagen diseases and malignant exophthalmos are

all characterized by certain rather pathognomonic alterations in the interstitial tissues, the capillaries and the supporting matrix of skin and mucous membranes. It is upon these that the steroid may act directly. It seems likely that cortisone exerts its effects in a similar manner, as the doses of the latter necessary to effect a change are far beyond the range necessary to substitute for the supply normally made available from the adrenal gland. In this connection it should be emphasized that pregnenolone exerts its beneficial action at dosage levels from which one need not fear any toxic reactions of any kind. Although it is indeed less active in a majority of cases than is cortisone, its complete freedom from untoward systemic effects justifies its further extended trial in those diseases for which it has been here found helpful.

SUMMARY

1. Fifty-nine patients with various types of clinical disorders have been treated with pregnenolone. These include instances of arthritis, lupus erythematosus, malignant exophthalmos, scleroderma, psoriasis and Addison's disease.

2. Six out of 11 patients with rheumatoid arthritis were moderately or markedly improved by pregnenolone. Seven out of 13 patients with hypertropic osteoarthritis were also moderately or markedly improved. A case of acute nonspecific arthritis and a case of Still's disease were markedly benefited by the drug.

3. Pregnenolone proved to be markedly effective in the treatment of chronic lupus erythematosus, malignant exophthalmos, scleroderma and psoriasis.

4. Pregnenolone was without effect in the treatment of Addison's disease.

5. The daily dose of pregnenolone, the duration of treatment and the possible mechanisms of action of the drug have been discussed.

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STUDIES OF URINARY 17-KETOSTEROID EXCRETION BY MEANS OF A NEW MICRO-CHROMATOGRAPHIC FRACTIONATION PROCEDURE*

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I. INTRODUCTION

IN THE extensive studies reported by other workers (1–8) and in previous communications from this laboratory (9–11), it is clearly evident that neutral urinary 17-ketosteroid excretion may undergo important qualitative as well as quantitative alterations under certain circumstances. At times definite qualitative changes occur in the absence of significant variations in total urinary 17-ketosteroid output. Hence, determination of the composition of steroids excreted in the urine may be expected to yield considerable information in addition to that provided by simple measurements of total output.

To date most of the urinary steroid fractionation studies have been performed with the aid of extensive procedures involving the use of large pooled specimens of urine and the availability of highly trained personnel. These factors rendered these procedures unsuitable for use in most small clinical and research laboratories. In a recent publication, Dingemanse, Veld and Laat described a simplified procedure for the chromatographic partition of urinary 17-ketosteroids into a number of fractions (12). Preliminary experience with this procedure in this laboratory indicated that a method of this type might serve as a satisfactory substitute for detailed fractionation studies under most circumstances. At the same time, it appeared that the procedure reported might be improved by certain modifications.

The present paper describes a relatively simple method for fractionating 0.3-mg. samples of neutral urinary 17-ketosteroids into 40 separate fractions. The potential usefulness of this procedure in clinical and physiologic problems is illustrated by selected studies.

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II. METHOD

A **Reagents:**[1]

1. *For hydrolysis and extraction.*

Technical hydrochloric acid
Carbon tetrachloride
Ethyl ether (Mallinckrodt analytical reagent)
Toluene
1.0 N aqueous sodium hydroxide solution

Carbon tetrachloride and ether may be recovered after use and redistilled in an all-glass apparatus. Carbon tetrachloride is stored in dark bottles and ether in metal containers.

2. *For Girard reaction.*

Girard's reagent P (Edwal Laboratories, Inc.)
Glacial acetic acid
10% Aqueous sodium hydroxide solution.

U.S.P. chloroform (Dow Chemical Co.), distilled in an all-glass apparatus and stored in dark bottles.

Sulfuric acid (C. P., Baker's special)
0.1 N aqueous sodium hydroxide solution

3. *For colorimetric assay*

Absolute ethanol (U. S. Industrial Chemicals, Inc.). Certain other brands have given excessively high blank values.

Potassium hydroxide, reagent pellets (Merck and Co., Inc.) A 2.5 N alcoholic solution is made up at least at weekly intervals as previously described (13).

m-Dinitrobenzene (Eastman Kodak Co.), purified by recrystallizing from methyl alcohol after treatment with Norit A. A solution containing 11.6 mg. per ml. is made up in absolute ethanol at weekly intervals.

95% Ethanol (U. S. Industrial Chemicals, Inc.)

4. *For chromatographic fractionation.*

Anhydrous aluminum oxide "according to Brockman" (Merck and Co., Inc.). Lot 41333 was used in the standardized technique. Alumina, which appears to lose activity upon repeated exposure to air, must be kept free from contamination with acid fumes and moisture. This may be done by replacing the cap of its container with a large rubber stopper in which is inserted a glass tube of small bore (Fig. 1, Section A). A 25-cm. length of thin-walled, small-bore rubber or plastic tubing is attached to the glass tube and clamped off with two screw-clamps placed about 15 cm. apart. The stopper and connecting parts are sealed in place with paraffin. This arrangement makes it possible to withdraw small portions of alumina without contaminating the remainder.

Absolute methanol (acetone-free). Methanol is dried by refluxing for three hours with magnesium turnings, and is distilled into oven-dried, glass-stoppered flasks.

Benzene (thiophene-free, as dispensed in 5 lb. bottles).

Solvent mixtures: The following eluting solutions of methanol in benzene are freshly prepared immediately before use. Storing large volumes of these solvents is unsatisfactory due to an apparent change in methanol concentration on standing. a) 0.05% methanol in benzene, b) 0.10% methanol in benzene, c) 0.20% methanol in benzene, d) 0.50% methanol in benzene

Nitrogen, dry, 99.7% pure (Airco, Inc.)

[1] Reagents are C. P., Baker's, unless otherwise indicated.

B. Apparatus:

1. The micro-column is shown in Section B, Figure 1. The apparatus is acid-cleaned, thoroughly rinsed with distilled water and oven-dried before use.

2. An oxygen gauge type of reduction valve (Rego Type XL, 2) satisfactorily regulates the flow of nitrogen to the column.

3. Evelyn photoelectric colorimeter, with filters having maximum transmissions at 540 μ and 420 μ.

C. Analytic procedure

1. *Hydrolysis, extraction and purification of urinary 17-ketosteroids.*

a. Urine collection: Urines are collected without preservative. The sample to be analyzed should contain at least 0.5 milligram of 17-ketosteroid.

b. Hydrolysis and extraction: Three approximately similar methods have been used: (a) separate hydrolysis and subsequent extraction with carbon tetrachloride, (b) same as (a) but using ethyl ether as a solvent, and (c) simultaneous hydrolysis and extraction with toluene.

In procedure (a) the urine is boiled with 15 volumes per cent of hydrochloric acid for ten minutes under a reflux condenser and cooled rapidly. After cooling, it is transferred either to a continuous extraction apparatus of the Hershberg-Wolfe (14) type or to a separatory funnel, and extracted with carbon tetrachloride. If the separatory funnel is used, the hydrolyzed urine is extracted four times with 15 volumes per cent of solvent. The combined extract is evaporated to a convenient volume and washed four times with 25-ml. lots of 1 N sodium hydroxide and four times with 25-ml. lots of water. Procedure (b) is the same as (a), except that the hydrolyzed urine is extracted in a separatory funnel with ether. In procedure (c) the Astwood (15) method of simultaneous hydrolysis and extractions with toluene was adapted for small volumes of urine which are hydrolyzed with 15 volumes per cent of hydrochloric acid by boiling on a Wood's metal bath for twenty minutes under a reflux condenser. After cooling, the aqueous layer is discarded, and the toluene is washed three times with 10-ml. lots of 1 N sodium hydroxide and three times with 15-ml. lots of water.

All washed extracts are evaporated to dryness with the aid of suction on a steam bath. Residual traces of solvent are removed by the addition and subsequent evaporation of small lots of methanol. The nonvolatile residue (crude, neutral 17-ketosteroid fraction) then is separated into ketonic and nonketonic fractions as outlined in the next paragraphs.

c. Ketonic separations: To 20 mg. or less of dry, crude, neutral 17-ketosteroid material, 200 mg. of Girard's reagent P and 0.5 ml. of glacial acetic acid are added. The flask containing this mixture is stoppered with aluminum foil and heated for three minutes in a boiling water bath. It is rotated occasionally during the heating period to insure complete mixing of the contents. At the end of this period the flask is chilled in an ice bath. The contents are then transferred quantitatively to a separatory funnel with a total of 40 ml. of ice-cold distilled water. After the addition of 3 ml. of 10 per cent sodium hydroxide solution, the resultant mixture is extracted three times with 20-ml. portions of chloroform. The chloroform extracts are combined and backwashed once with 60 ml. of distilled water. This chloroform extract, containing the nonketonic fraction, is discarded. One ml. of concentrated sulfuric acid is added to the 60-ml. water backwash, which is then combined with the aqueous phase remaining after extraction of the nonketonic fraction. After the addition of 20 ml. of chloroform, the mixture is

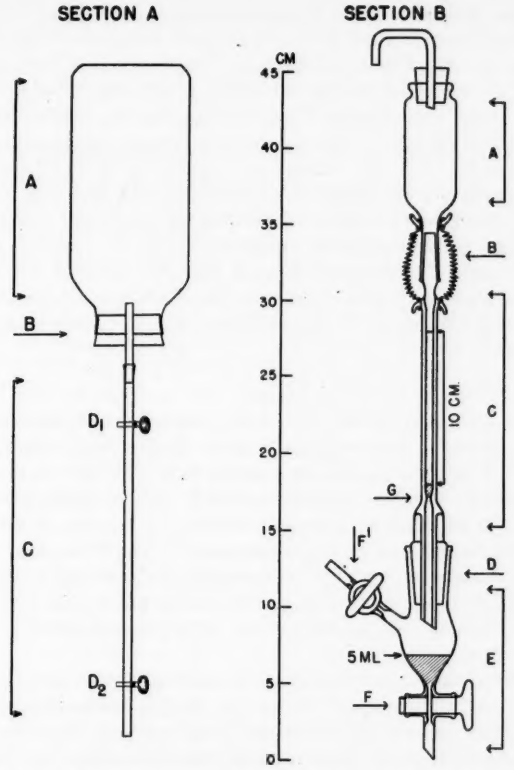

FIG. 1. *Section A.* **A** is a stock alumina bottle with its cap replaced by a large rubber stopper, **B,** in which is inserted a 5-cm.-long × 5-mm.-wide glass tube. To the glass tube is attached a 25-cm. length of Tygon tubing, **C**. Connecting parts are waxed over completely. Two screw-clamps, $\mathbf{D_1}$ and $\mathbf{D_2}$, are placed about 20 cm. apart on tube **C,** which is filled with alumina by releasing clamp $\mathbf{D_1}$ just prior to packing the chromatographic column.

Section B. Chromatographic adsorption micro-apparatus. **A** is a 50-ml. glass reservoir fitted at the top with a rubber stopper containing an inlet tube for nitrogen gas.* **B** is a ground-glass joint held in place by two springs. **C** is a heavy-walled glass column of True-bore tubing with an exact inside diameter of 4 mm. **D** is a ground-glass joint. **E** is a pear-shaped funnel of 20-ml. capacity, calibrated at 5 ml. It is fitted with two ground-glass stopcocks, **F** and **F′**. **F′** acts as an air vent and may be used to stop the flow of solvent when pressure is released at **A**. The complete apparatus was made by Macalaster Bicknell Company, Cambridge, Massachusetts.

* The nitrogen is used to push the various solvents through the column of aluminum oxide. A satisfactory flow rate is obtained when the oxygen gauge pressure reading is adjusted to about 2.2 liters per minute. Adjustments to this pressure are made after the flow of N_2 through the column has been initiated at the lowest rate possible. At the adjusted rate, a 5-ml. fraction is collected in three minutes.

shaken briefly and allowed to stand at room temperature for two hours.[2] The resultant acid-hydrolyzed aqueous mixture is then extracted with the chloroform present and with three additional 20-ml. lots of chloroform. The chloroform extracts containing the ketonic material are combined, washed once with 10 ml. of 0.1 N sodium hydroxide and three times with 10-ml. lots of water. These washings are discarded. The washed ketonic chloroform extract is evaporated to approximate dryness on the steam bath with the aid of suction. Residual traces of solvent are removed by the addition and subsequent evaporation of small lots of methanol. All residues are then dried over anhydrous calcium chloride in a vacuum desiccator for at least forty-eight hours.

The dried residues are dissolved in benzene and stored in oven-dried, glass-stoppered bottles. The amount of benzene used depends upon the amount of steroid present; an attempt is made to add such an amount that the resultant solution will contain about 0.5 mg. of 17-ketosteroid per ml. The exact concentration is determined by colorimetric assay as described below.

2. *Colorimetric assay.*

An accurately measured 0.1-ml. or 0.2-ml. aliquot of the benzene solution of 17-ketosteroids is transferred to a colorimeter tube. The benzene is evaporated with the aid of suction by warming the tube in a steam bath. The dry residue is dissolved in 0.1 ml. of absolute ethanol, to which are added 0.1 ml. of ethanolic m-dinitrobenzene solution and 0.1 ml. of ethanolic potassium hydroxide solution. A blank solution containing no steroid is prepared in the same manner. The tubes are rotated gently to insure complete mixing and then are immersed in a darkened, constant temperature water bath for eighty minutes at 25° C. $\pm$0.05°. At the end of this incubation period the contents of each tube are diluted with 10 ml. of 95 per cent ethanol and mixed by inverting the tube once.

Colorimetric readings are made with green and blue filters, having maximum transmission at 540 μ and 420 μ respectively, in the Evelyn photoelectric colorimeter after adjusting the galvanometer to 100 with the blank in place. The green filter reading is referred to a calibration curve or table made with crystalline pure androsterone, isoandrosterone or dehydroisoandrosterone. The resultant value is called the "uncorrected" 17-ketosteroid value. It indicates approximately the quantity of 17-ketosteroid present in the sample analyzed. This crude value is "corrected" for errors of overestimation due to interfering chromogens by application of a color correction equation based on the relation between green and blue filter galvanometer readings as described elsewhere[3] (16). The "corrected" assay value is used in determining the concentration of 17-ketosteroids in the benzene solution to be fractionated and hence in determining what volume of this solution should be placed on the chromatographic column.

The foregoing procedure is modified as follows to facilitate the determination of the 17-ketosteroid content of each of the 41 fractions obtained by elution of the column as described below. Equal volumes of the reagents (ethanol, m-dinitrobenzene and potassium hydroxide) are combined and mixed immediately before use. To a blank tube and to each tube containing a dry residue, 0.3 ml. of the mixture is added. The tubes are rotated and incubated as described above. Following incubation, the contents of each

[2] Fractionation patterns of 17-ketosteroids which were hydrolyzed for two and for twenty hours were nearly identical.

[3] There was a typographical error in this report. On page 211, in the second and third lines from the bottom of the page E_G:E_B should have read E_B:E_G.

tube are diluted with 10 ml. of 95 per cent ethanol, mixed, and read as above. The eluates are usually assayed in three lots of 14 each with a freshly prepared reagent mixture for each lot. The galvanometer readings obtained are referred to the calibration curve mentioned earlier.

3. *Chromatographic fractionation.*

a. Preparation of column (Fig. 1). A plug of alkali-free glass wool[4] is inserted at the constriction (letter G, section B). The Tygon tubing of the stock bottle of alumina (section A) is attached to the upper end of chromatographic tube C (section B). Clamp D_2 is opened, and, with gentle tapping to insure even distribution, alumina is allowed to run into the glass tube (C) until a column exactly 10 cm. long has accumulated. The clamp is closed, and glass tube C is disconnected and attached to funnel E. Solvent reservoir A is set in place and secured to the column by means of wire springs. Benzene (5 ml.) is percolated through the column under nitrogen pressure until the upper level of this solvent is within 2 to 3 cm. of the top layer of alumina.[5] The benzene which has accumulated in funnel E is discarded.

The surface of the alumina is now smoothed by removing the solvent reservoir A, stirring the surface slightly with the tip of a finely drawn-out dry, glass rod and gently tapping the column once or twice. The reservoir A is replaced and enough benzene is added to bring the fluid level to the top of the ground-glass joint B.

b. Fractionation. An aliquot of the previously assayed benzene solution containing 0.3 mg. of urinary 17-ketosteroids is diluted to 5 ml. with benzene in a dry test tube of 10-ml. capacity. The solution is transferred quantitatively to reservoir A with the aid of an additional 1 ml. of benzene. The solution, totaling 7 ml., now is percolated through the column of alumina, slowly under pressure, the percolate being collected directly into the first of a series of 41 photoelectric-colorimeter tubes. When the surface of the initial 17-ketosteroid-containing aliquot of benzene has fallen to a point about 1 cm. above the surface of the alumina, pressure is discontinued, and solvent mixture "a" is added to reservoir A; pressure is then resumed. Each 5 ml. of eluate is collected in a photoelectric-colorimeter tube when the level of the calibration mark of funnel E is reached. The process is repeated with solvent mixtures "b," "c," "d," and finally with absolute methanol, until elution is complete. The total amount of each solvent percolated through the column and the total number of fractions collected follows:

Solvent	No. of 5-ml. eluate fractions	Total ml. of eluate
Benzene (containing 17-KS)	(1)	7
Solvent mixture *a*	9	45
Solvent mixture *b*	19	95
Solvent mixture *c*	5	25
Solvent mixture *d*	5	25
Absolute methanol	2	10

[4] Fiberglass, pyrex, alkali-free (Corning glass).

[5] The alumina must be kept covered with solvent at all times. If allowed to dry, the column of alumina develops channels which interfere with even percolation of the solvent.

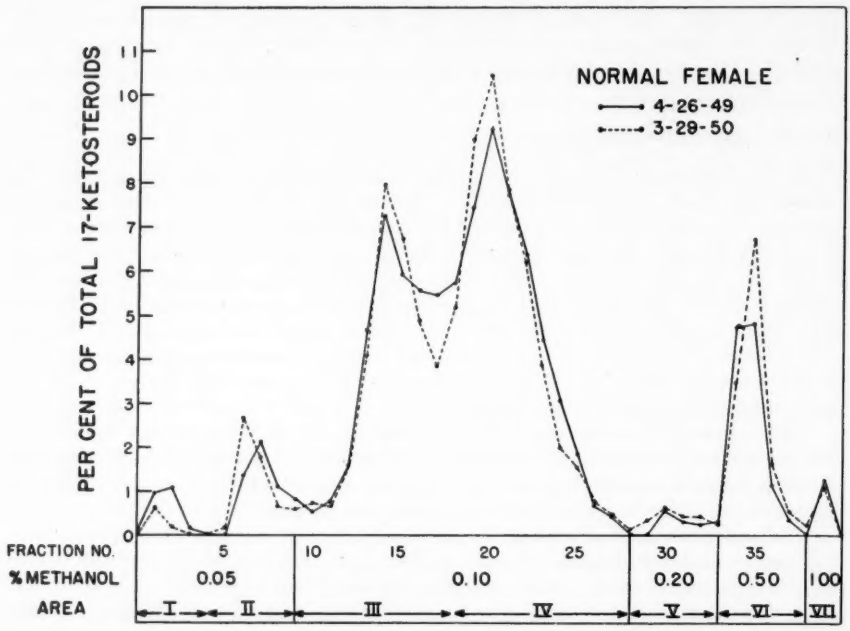

FIG. 2. *Duplicate chromatographic separations of neutral urinary 17-ketosteroids on equal aliquots of a single normal female urine extract.* One curve was obtained eleven months after the other. Each point indicates the per cent of the total 17-ketosteroids eluted (left-hand ordinate) which was recovered in the individual eluate fraction indicated by number along the abscissa. The abscissa also indicates the composition of the solvents used for elution as indicated in the text. The areas indicated at the bottom of the abscissa are arbitrarily intended to conform approximately to the areas subtended by the curve peaks. Note that the two curves correspond quite closely.

Each eluate fraction is evaporated to dryness in a boiling water bath with the aid of suction. The nonvolatile residue, which has a tendency to creep up the sides of the test tube, is rinsed down with about 1 ml. of absolute, acetone-free methanol and is dried with suction.

c. Calculation of results. The 17-ketosteroid content of each eluted residue, expressed as mg. per fraction, is measured by colorimetric reaction as described previously. The total recovery is determined by adding the values of the 41 eluates. Each fraction is then recalculated as "per cent of total 17-ketosteroids" recovered.

III. RESULTS AND COMMENTS

The first portion of this section is concerned with data indicating the reliability of the chromatographic fractionation procedure. The second portion deals with preliminary observations on the physiologic and clinical usefulness of this type of urinary 17-ketosteroid fractionation study.

A. Basic methodologic observations.

1. *Duplicability of results.*

Figure 2 presents the results obtained after chromatographic separation of two equal aliquots of a single, normal female urine extract. These observations, which are representative of other similar studies, indicate that the results obtained by a single analysis can be duplicated satisfactorily.

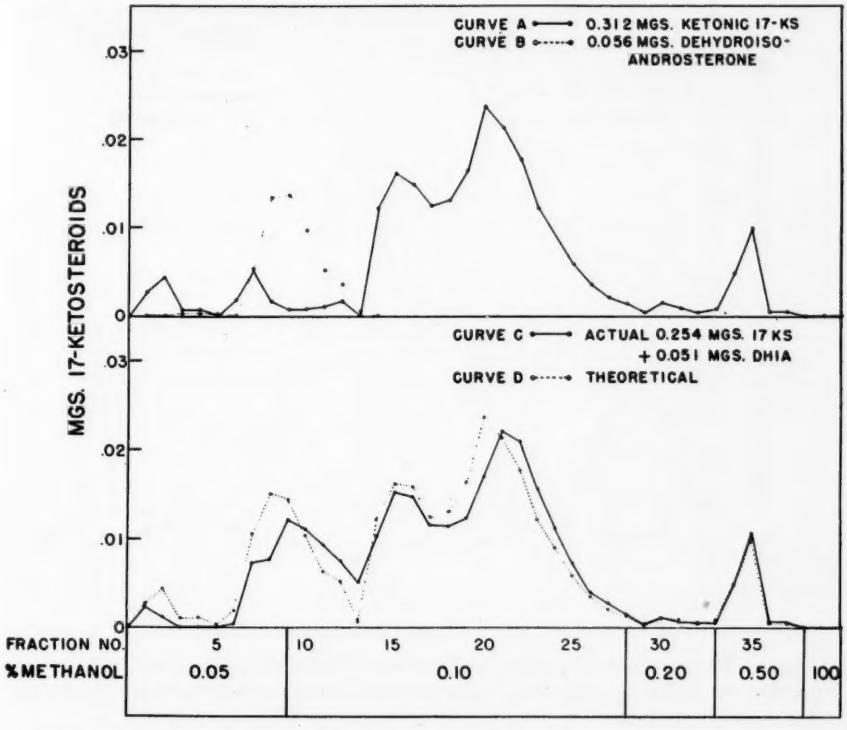

FIG. 3. *Effect of altering composition of mixture of urinary 17-ketosteroids by substitution of known amount of crystalline pure 17-ketosteroid.* The upper section of the figure gives the results obtained by analysis of measured amounts of (A) a ketonic fraction of neutral urinary steroids and (B) dehydroisoandrosterone. The lower section of the figure presents (C), the actual results obtained after substituting a known amount of dehydroisoandrosterone for an approximately equal amount of mixed urinary 17-ketosteroids, and (D) the theoretical results expected, on the basis of Curves (A) and (B), from such a substitution. Note that the actual and theoretical curves correspond reasonably satisfactorily. The values of Curve (A) have been reduced by $\frac{1}{6}$ of the actual milligrams per fraction, in order that Curve A may be on a comparable scale with Curves C and D.

2. *Consistency of behavior of 17-ketosteroids on chromatographic column.*

Figure 3 presents studies designed to find out whether or not the introduction of a steroid which produces a peak in one area causes significant changes in other areas of the chromatographic curve. The experiment consisted in three chromatographic analyses. In the first of these, an 0.312–mg. aliquot of an urinary 17-ketosteroid sample (Curve A) and in the second,

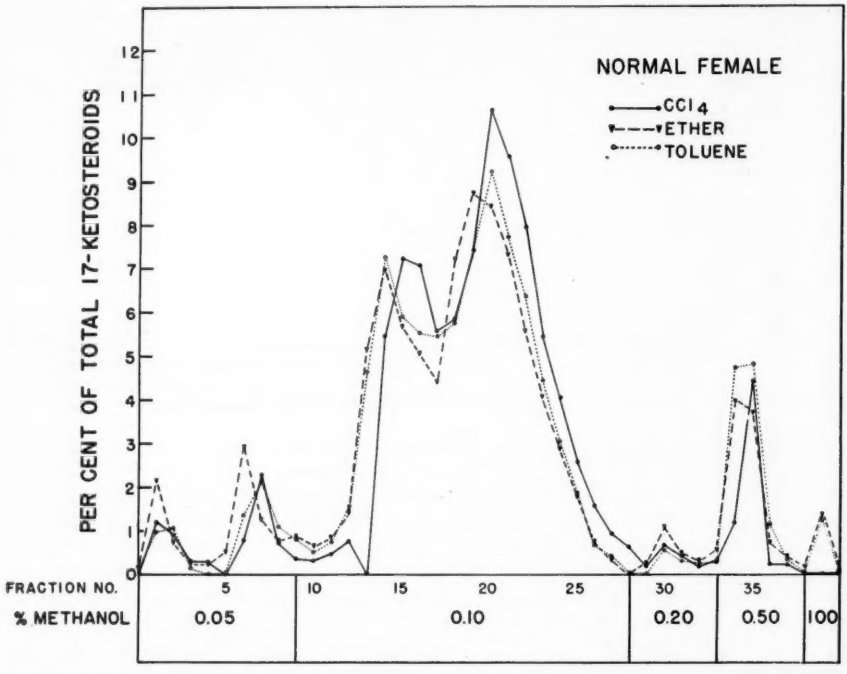

FIG. 4. *Effect of various hydrolysis and extraction procedures upon character of chromatographic fractionation curves.* The nature of the procedures used is indicated in the text.

0.056 mg. of crystalline dehydroisoandrosterone (Curve B) were subjected to chromatographic fractionation. In the third experiment, 0.051 mg. of dehydroisoandrosterone and another 0.254-mg. aliquot of the urinary 17-ketosteroid sample represented by Curve A were mixed and subjected to chromatographic separation (Curve C). Curve D is the theoretical sum of Curves A and B. It was found by adding to 0.254/0.312 of each value of Curve A 0.051/0.056 of each value of Curve B. It can be seen that Curves C and D correspond reasonably well. In like manner agreement between actual and theoretical curves has been obtained in a similar experiment in

which 0.15 mg. of etiocholanolone was substituted for an equal amount of urinary 17-ketosteroid mixture.

These observations suggest that qualitative differences in the composition of 17-ketosteroid mixtures do not markedly affect the behavior of individual 17-ketosteroids in the chromatographic fractionation procedure.

3. *Effect of hydrolysis and extraction procedure upon character of chromatographic fractionation curve.*

Figure 4 shows 3 curves obtained in an experiment wherein aliquots of a pool of normal adult female urine were hydrolyzed with acid and extracted

TABLE 1. BEHAVIOR OF SELECTED, CRYSTALLINE 17-KETOSTEROIDS IN THE CHROMATOGRAPHIC FRACTIONATION PROCEDURE

No.	Substance fractionated	Melting point	Amount used	Fractions in which substance appeared	Fractions containing largest amount
		° C.	mg.	Number	Number
1	Chlorodehydro-isoandrosterone	155–158	.2	0 to 3	1
2	Δ^4 Androstenedione 3, 17	172–173	.2	6 to 9	7
3	Isoandrosterone	174–176	.14	6 to 14	8
4	Dehydroisoandrosterone	139, 152	.1	6 to 14	8–9
			.06	6 to 14	8–9
5	Androsterone	182–183	.14	11 to 20	15
6	Etiocholanolone	147–148	.3	15 to 28	20
			.15	15 to 28	20

with either carbon tetrachloride, ethyl ether or toluene as described previously. Since the results obtained on urine extracts prepared in these various ways were nearly identical, it is concluded that these three methods of hydrolysis and extraction may be considered interchangeable.

4. *Position of known compounds in chromatographic separation curves.*

Table 1 indicates where certain crystalline 17-ketosteroids appear upon being subjected to the standardized fractionation procedure described. These findings are in keeping with those obtained by others using similar aluminum oxide columns (5, 7, 12, 17, 18).

5. *Comments.*

The analytic procedure described here must be followed closely if similar results are to be obtained. Among the factors specifically requiring control, the following appear to be most important.

a. *Activity of aluminum oxide reagent.* The adsorbent activity of alumina remains quite constant if it is stored in a tightly sealed container at room temperature. Exposure to air and especially to moisture can result in a marked loss of activity. When this occurs, there is a tendency for 17-ketosteroids to be eluted more easily. This is reflected by a shift to the left and merging of the chromatographic fractionation curve peaks. Similar changes in alumina activity and similar shifts in curve peaks are produced by heating samples to 300° C. or more. These facts form the basis for the direction concerning storage of alumina, drying of glassware and reagents used for elution, and use of dry nitrogen rather than air pressure.

b. *Size of aluminum oxide column and load of 17-ketosteroids.* Approximately 1 Gm. of aluminum oxide is required to form the 10-cm.-long by 4-mm.-diameter column used in these studies. Under conditions of a constant 0.3-mg. load of urinary 17-ketosteroids, a 0.3-cm. deviation in the length and small deviations in diameter can result in marked alterations in curve patterns. Hence, it appears important to use glass tubing of constant, known bore. If the directions for filling the tube with alumina are followed reasonably closely, no difficulties should be encountered in making a column of constant characteristics.

Under the conditions of the constant aluminum oxide column described here, variations in the quantity or load of 17-ketosteroids placed on the column also can lead to marked changes in the shape of fractionation curves. If the recommended load of 0.3 mg. of 17-ketosteroids is increased by 30 or more per cent, there results the same tendency to early elution of 17-ketosteroids and merging of curve peaks noted when the adsorbent activity of aluminum oxide is reduced by exposure to moisture. Hence the recommendation that an approximately constant amount of material be used in each fractionation. In this connection it is noteworthy that nonketonic urinary substances, by overloading the aluminum oxide, also can cause important shifts in urinary 17-ketosteroid fractionation curves. Fortunately these nonketonic substances are largely removed in the ketonic separation with Girard's reagent.

A decrease in the quantity of 17-ketosteroids placed on the column is without important effect upon the elution pattern. However, considerable reduction in the quantity of 17-ketosteroids subjected to chromatographic separation so decreases the amount of 17-ketosteroid material appearing in individual eluate fractions that accurate colorimetric assay of each fraction becomes impossible. Satisfactory results usually are obtained if at least 0.27 mg. of 17-ketosteroids is fractionated.

B. Clinical observations.

The following paragraphs present sample observations on 1) normal

individuals, 2) patients with adrenal cortical virilism, 3) patients suffering from nonendocrine conditions including cancer, and 4) the effect of ACTH administration:

1. *Normal individuals.* Figure 5 gives the absolute ranges obtained by chromatographic fractionation of the 17-ketosteroids excreted by 6 nor-

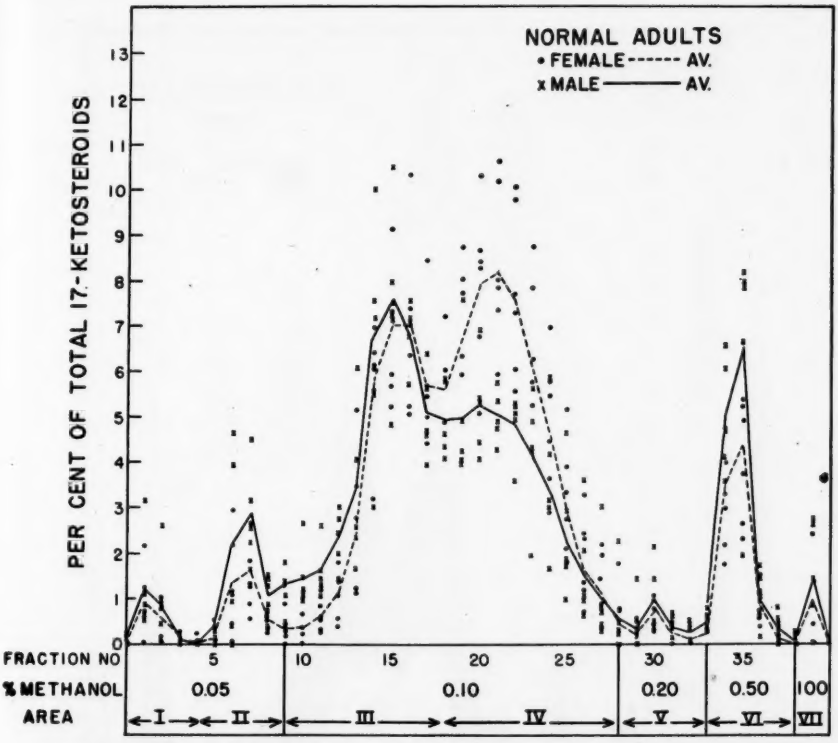

FIG. 5. *Observations on normal adults.* The abscissa and ordinate of this diagram correspond to those of Figure 2. The interrupted and solid curves indicate average values for women and men, respectively. Average recoveries per area are: for females, I—1.7%, II—4.0%, III—35.7%, IV—47.2%, V—1.6%, VI—8.9%, VII—0.9%; and for males, I—2.4%, II—7.7%, III—39.7%, IV—33.6%, V—2.5%, VI—13.2%, VII—1.4% of total 17-ketosteroids recovered.

mal men and 4 normal women. Six definite peaks appearing approximately in Fractions 2, 6–7, 14–15, 20–21, 35 and 39 are evident. In addition, there is a less definite peak at Fraction 30. Although the patterns shown for the two sexes are generally similar, they differ in two or three areas. Men appear to excrete more material which is eluted in Fractions 5–12 and less

that appears in Fractions 19–24, than women. The differences in Fractions 34 and 35 may also be significant.

2. *Adrenal cortical virilism.* Figure 6 portrays the curves obtained on 6 patients, aged 6 to 19 years, with adrenal cortical virilism due to bilateral adrenal cortical hyperplasia. In 5 patients the disease appeared to be of the classic congenital type with urogenital sinus formation in the female pa-

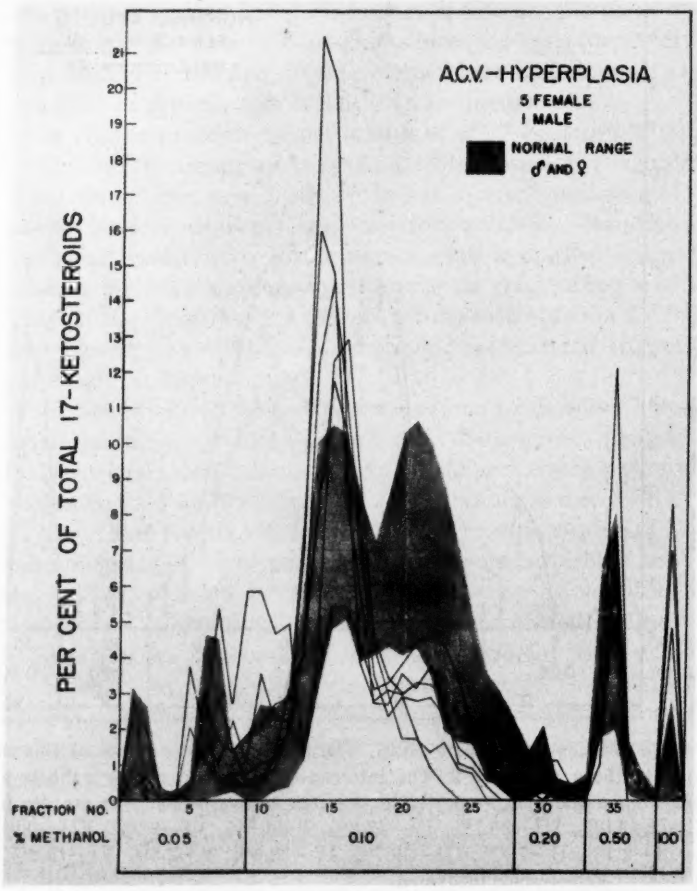

FIG. 6. *Observations on 6 patients, 6 to 19 years of age, with adrenal cortical virilism (A.C.V.) due to bilateral adrenal cortical hyperplasia.* In 5 of these patients the disease was considered congenital; in 1 it became clinically manifest when the patient was about 5 years old. The shaded area represents the normal range shown in Figure 5. Results are expressed as per cent of total 17-ketosteroids excreted. Total output values according to the ages of the patients were: for the 6-yr.-old, 11.4 mg; 9 years., 7.9 mg.; 9 yrs., 131 mg.; 12 yrs., 27 mg.; 13 yrs., 66 mg.; and 19 yrs., 43 mg.

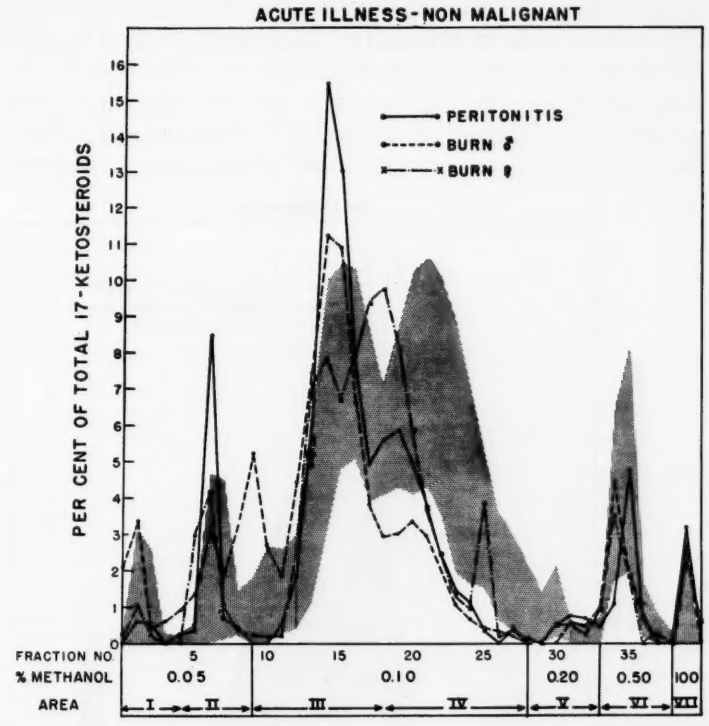

FIG. 7. *Observations on a boy, aged 14, with acute peritonitis; and on a male, aged 20 and a woman, aged 30, with extensive burns of the skin.* The design of this figure is the same as that of Figure 6. The total 17-ketosteroid output of the peritonitis patient was 0.14, of the burned male 35.4, and of the burned woman 11.3 mg. per 24 hours.

tients. In 1 of the females, there was hypertrophy of the clitoris dating back to early childhood, but no other urogenital malformation. All patients had abnormally high total 17-ketosteroid output values.

Note that none of these patients had normal curves. The 5 individuals having congenital adrenal cortical virilism are represented by the curves showing abnormally high values in the area defined by fractions 13–15. The female with virilism, but without a urogenital sinus is represented by the curve seen to be abnormally high in the zone defined by Fractions 9–12 and low-normal in Fractions 13–15. All the patients tended to have abnormally low values in Fractions 17–25. One of the subjects had an abnormally high value in Fraction 35 and three had high values in Fraction 39.

3. *Acute illness.* Figure 7 presents the findings obtained on an adolescent boy with peritonitis secondary to intestinal obstruction and on 2 adults

with extensive thermal burns. Note that the curves shown fall outside the limits of normal in Areas II, III and IV. Especially in Areas III and IV they resemble a number of those given in Figure 6.

4. *Malignant disease.* In Figure 8 it is seen that the chromatographic fractionation curves obtained on patients with neoplastic disease tend to be abnormal in one or more respects. The curve representing the inflammatory

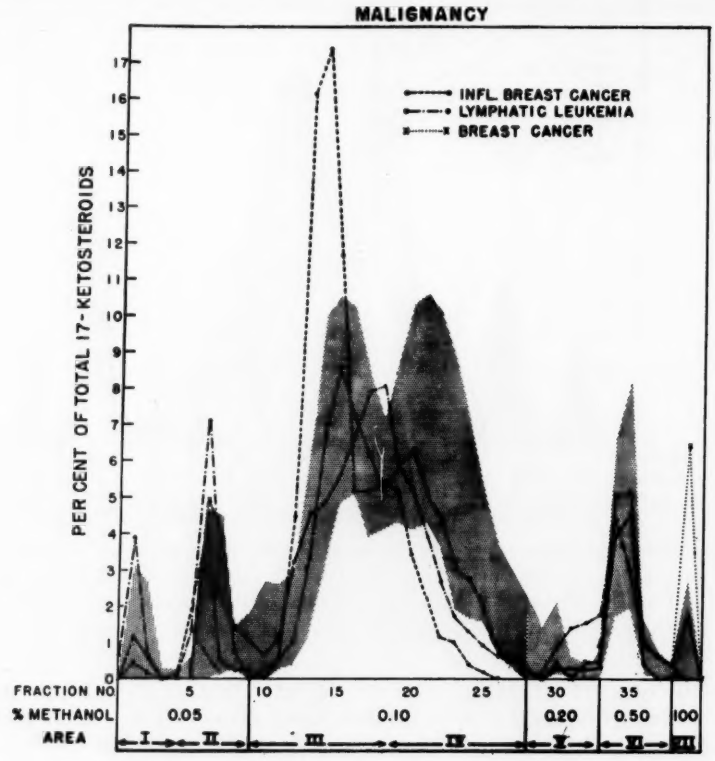

FIG. 8. *Observations on 3 adults with malignant disease.* The design of this figure corresponds to that of Figure 6. The total output of 17-ketosteroids per day was: for the inflammatory breast cancer patient, 10.2 mg.; for the lymphatic leukemia patient, 3 mg.; and for the breast cancer patient, 6.3 mg.

breast cancer patient is somewhat like those shown in Figures 6 and 7. The other 2 curves are dissimilar to any observed heretofore.

5. *Effect of ACTH administration.* Figure 9 gives information concerning changes in the output of various urinary 17-ketosteroid fractions following treatment with Armour adrenocorticotropic hormone. Note that the chromatographic curve patterns observed following administration of ACTH

do not correspond in shape either to the control curves obtained on these 2 patients or to those found for normal subjects (Figure 5) . It would appear that ACTH stimulates the production of unusually large amounts of those

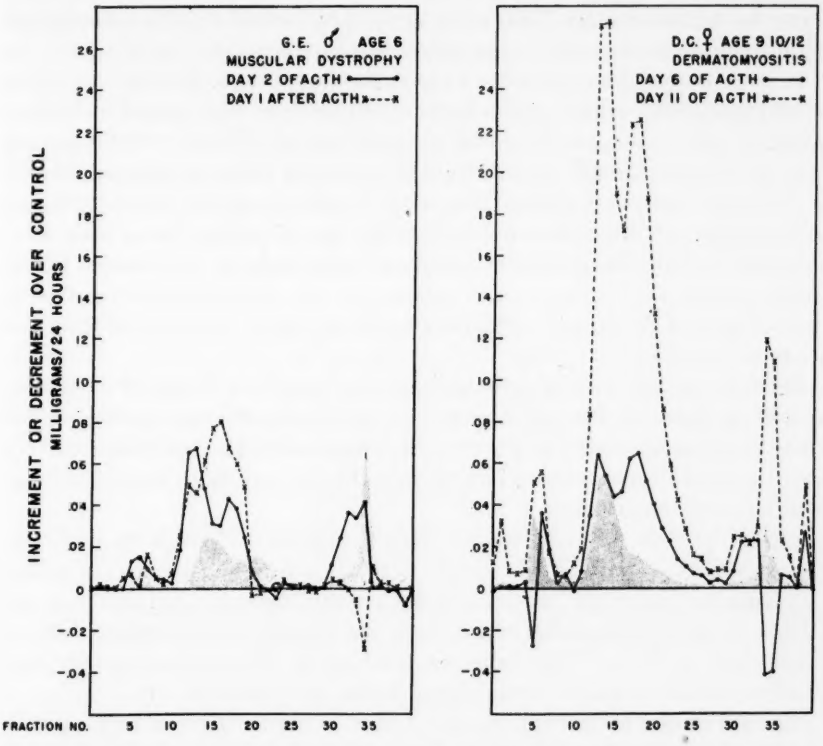

FIG. 9. *Increments and decrements from control values in the urinary output of various urinary 17-ketosteroid fractions following ACTH administration.* In this figure the upper margin of the shaded area indicates the absolute milligrams of 17-ketosteroid appearing in each fraction per control 24-hour urine specimen (left-hand ordinate). The curves give the absolute changes from control values, in milligrams per 24 hours, observed following the administration of ACTH. The increment and decrement values are given by the scale along the left-hand ordinate. Patient G. E. was given 22 mg. and patient D. C. was given 12.5 mg. of Armour standard ACTH every six hours.

types of 17-ketosteroid which appear in Fractions 10–18 and may either inhibit or augment the excretion of substances appearing in Fractions 6, 34 and 35. Armour ACTH does not appear to alter the rate of elimination of 17-ketosteroids of the types appearing in the region of Fraction 25 to a major extent.

COMMENTS

The foregoing preliminary clinical observations suggest that normal individuals of each sex may be expected to show chromatographic fractionation patterns of fairly constant character. It would appear, however, that separate normal standards should be developed for males and females. More detailed information concerning adolescent patterns are needed.

It is clear that patients with adrenal cortical disease excrete a mixture of 17-ketosteroids which differs in composition from that found in normal urine. It also is evident that the composition of urinary 17-ketosteroids may become abnormally altered by such primarily nonendocrine conditions as infection, burn and malignancy. This finding suggests that adaptation to stress leads to alterations not only in the rate of production of total steroids, but also to changes in the nature or intermediary metabolism of the steroids elaborated. These observations are in essential agreement with those reported by others employing more extensive methods of fractionation (5, 19, 20).

Since abnormal chromatographic patterns have been observed in females as well as males, it does not appear that the changes noted are dependent upon the presence either of the ovaries or the testes. Rather it seems likely that the alterations seen are largely referable to variations in adrenal cortical hormone metabolism.

It is of interest that patients having a neoplasm may show an abnormal chromatographic pattern whether or not they are obviously sick. In an observation not recorded here, a normal chromatographic pattern was obtained on a patient with proved, localized cancer of the breast, but no other signs of illness. This indicates that cancer does not necessarily lead to alterations in urinary steroid composition as measured here.

The adrenocorticotropic hormone studies of Figure 9 are in keeping with Dobriner's observation that large doses of ACTH cause qualitative as well as quantitative alterations in urinary 17-ketosteroid excretion (20). They also add support to the idea that Armour ACTH may differ from human, endogenous ACTH in its effect upon the adrenal cortex (21). Stated in other words, the steroid mixtures excreted in response to ACTH administration appear to differ appreciably from those appearing prior to ACTH treatment. Moreover, they do not give rise to patterns identical with those seen in normal subjects (Fig. 5). On the other hand, the fact that ACTH may at one time prompt a decrease and at another an increase in excretion of certain types of 17-ketosteroid (*see* Fractions 30–35, Fig. 9) suggests that adrenal cortical responsiveness to ACTH may undergo qualitative changes under certain circumstances. This thesis is supported by observations which indicate that ACTH administration causes the adrenal cortices of

infants to secrete more "11–17-oxycorticosteroids" and less 17-ketosteroid precursors than in older children and adults (21).

SUMMARY AND CONCLUSIONS

A relatively simple, microchromatographic fractionation procedure for separating 0.3 mg. of neutral, urinary 17-ketosteroids into 40 separate fractions is described. A three-hour collection of ordinary adult urine contains sufficient 17-ketosteroid for this fractionation. This method has given satisfactorily reproduceable results over a period of two years.

The chromatographic fractionation curves obtained for normal adults are of quite constant character. They show 6 major peaks and 1 minor peak, each of which represents groups of 17-ketosteroids with different physicochemical properties. A slight, but seemingly definite, sex difference is apparent.

Abnormal curves have been obtained both for patients with adrenal cortical disease and for endocrinologically normal patients suffering trauma, infection or cancer. This suggests that adrenal cortical hormone metabolism may undergo qualitative changes not only under conditions of adrenal cortical pathology, but also in response to physiologic stress.

Acknowledgment

The authors are indebted to Dr. Lewis L. Engel of the Huntington Memorial Laboratories for his advice in the course of these investigations and to Dr. Ira Nathanson and Miss Gladys Eckman for their assistance in providing us with urinary extracts. The steroids used in these studies were generously provided by Dr. Erwin Schwenk of the Schering Corporation and the ACTH was generously supplied by Dr. John Mote of the Armour Laboratories.

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LATE RESULTS IN THE TREATMENT OF THYROTOXICOSIS WITH GOITROGENIC COMPOUNDS

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SINCE the first report by Astwood(1) in 1943 of the clinical use of thiourea in thyrotoxicosis, many goitrogenic compounds have been tried in this disorder. At present two thiouracil compounds, 6N propylthiouracil and methylthiouracil, are the most widely used by physicians in general practice. Such compounds have these advantages,—they are inexpensive, readily available, and tolerance or "fastness" to them does not develop. Because of their widespread use it seems desirable to report follow-up observations on a selected group of patients with unquestionable thyrotoxicosis treated with several goitrogenic substances and followed for intervals ranging from two to more than six years. This report deals with 46 such patients. Nineteen received thiouracil, 19 received propylthiouracil, 2 received aminothiazole, and 6 received thiouracil followed by propylthiouracil. All were selected for prolonged treatment because (a) the clinical picture suggested the probability of obtaining a sustained remission (mild to moderate severity of symptoms without marked thyroid enlargement), (b) because they presented postoperative recurrences of thyrotoxicosis, or (c) because they refused thyroidectomy. Initially the total daily dosage was divided into three equal parts. This was gradually reduced to a single daily maintenance dose as remission developed. The average daily initial dose of thiouracil was 0.7 Gm.; of propylthiouracil, 0.15 Gm.; and of aminothiazole, 0.6 Gm. The average daily maintenance doses were 0.1, 0.04 and 0.1 Gm., respectively. Data pertaining to age, sex distribution and types of goiter are shown in Table 1.

Whenever possible, patients were examined, leucocyte counts were made and basal metabolic rates determined at intervals of one to two weeks, until clinical remission was established. Thereafter, examinations were made at monthly intervals for approximately one year or until the withdrawal of treatment. In patients in whom the disease remained in remission, intervals between examinations were gradually lengthened to three months. Clinical remission was determined by the following criteria:

1. Reduction in basal metabolic rate to +15 per cent or less.
2. Disappearance of tachycardia.
3. Subsidence of subjective symptoms.

In 40 patients the disease remained in remission for periods varying from

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TABLE 1. AGE, SEX AND GOITER TYPES

	No. of patients
Age (years)	
10–20	5
21–30	9
31–40	15
41–50	9
51–60	7
61–70	1
	46
Sex	
Female	36
Male	10
	46
Goiter types	
Slight diffuse	40
Nodular	1
Postoperative recurrence	5
	46

seven to seventy-one months after withdrawal of medication. In 8 of these a relapse occurred from one to three times, either during or after the termination of treatment, before there was a sustained remission. Six other patients failed to show sustained remissions and are classified as failures. Of 8 patients who showed a relapse prior to sustained remission, 4 had had previous treatment. Among 32 patients who enjoyed sustained remissions without relapse, 20 had received previous treatment. Such treatment included stable iodine, external irradiation, subtotal thyroidectomy and short inadequate courses of thiouracil.

Data pertaining to the duration of treatment in relation to the length of post-therapeutic remissions are shown in Table 2. Of the 38 patients who showed a remission for one year or longer following withdrawal of treatment, 21 received treatment for total periods of less than twelve months. Among 22 patients in whom a remission was sustained for more than two years, 16 received treatment for total periods less than twelve months.

Comparison of the times required for full remission to appear after the beginning of treatment shows little difference between (a) the 8 patients who had a relapse one or more times before having a sustained remission, (b) the 6 patients classified as therapeutic failures, and (c) the 32 patients who had sustained remissions without preliminary relapses. The

average periods required were 1.8, 1.4, and 1.7 months in groups (a), (b), and (c) , respectively. The time elapsing between withdrawal and the appearance of a relapse in group (a) varied from one to sixteen months, the average being four. In group (b) the comparable periods were one and nine months, with an average of 2.3.

Among the 40 patients showing sustained remission, the thyroid enlargement eventually disappeared entirely in 25, decreased but remained palpable in 13, became nodular in 1, and remained unchanged in 1.

TABLE 2. RELATION OF TREATMENT TO REMISSIONS

Total duration of treatment	Duration of remission (post-therapeutic) in years							Total number of patients
	1 yr.	1–2	2–3	3–4	4–5	5–6	6–7	
Less than 6 mo.		2	2	1	3	3	1	12
From 6 to 12 mo.		3	3		3			9
More than 12 mo.	2	11	4	1	1			19
	2	*16*	*9*	*2*	*7*	*3*	*1*	*40*

Exophthalmos increased in 5 patients but in no instance was the increase accompanied by other evidence of the hyperophthalmopathic syndrome.

Seven patients had basal metabolic rates below −15 per cent at the time of the last examination, but none of these showed the clinical picture of myxedema.

In 3 of these patients mild symptoms of hypothyroidism developed at intervals of nine to twelve months after treatment was stopped. Spontaneous improvement occurred in 2; the other required small doses of thyroid substance for two months.

Manifestations of drug sensitivity or toxicity were observed in 7 patients (15.2 per cent). Among the 19 patients receiving thiouracil alone, agranulocytic angina developed in 1 and a transient mild papular dermatitis in 1. Among the 19 who received propylthiouracil, leucopenia (less than 4,000 leucocytes per cu.mm.) appeared in 2, dermatitis in 1 and vasomotor rhinitis in 1. One of the 2 patients receiving aminothiazole showed severe urticaria.

Data relating to the 6 patients classified as therapeutic failures are shown in Table 3. This group has been compared with the 40 patients showing sustained remission with respect to the following factors: age, sex, size and type of goiter, severity and duration of thyrotoxicosis, coexistent complications, previous treatment, dosage and duration of antithyroid medication, drug sensitivity, time required for initial remission and for the appearance of relapse after reduction or withdrawal of medication. This comparison yielded no information of prognostic value.

TABLE 3. THERAPEUTIC FAILURES

Patient	Age (yrs.)	Sex	Goiter type	Duration of disease	Duration of treatment	Time till relapse	Subsequent treatment	Result
M.B.	12	M	Diffuse	6 mo.	14 mo. propylthiouracil	2 mo.	Still under treatment with methylthiouracil	
S.S.	19	F	Postoperative recurrent toxic diffuse	18 mo.	2 yrs. propylthiouracil	2 mo.	I^{131}	Remission
E.W.	61	F	Diffuse	2 yrs.	2 yrs. thiouracil	1 mo.	X-ray (ineffective); subtotal thyroidectomy	Remission
T.W.	61	F	Nodular	2 mo.	32 mo. thiouracil 14 mo. propyl-thiouracil		$I^{131} \times 3$	Incomplete; remission; still toxic
J.G.	24	F	Diffuse	7 mo.	11 mo. and 10 mo.* thiouracil	1 mo.; 9 mo.*	I^{131}; thyroidectomy	Remission
S.F.	59	M	Diffuse	1 mo.	1 yr. and 1 yr.* thiouracil	2 mo.; 2 mo.*	$I^{131} \times 2$	Remission

* Two courses.

The relation of end-results to the type of goitrogen employed is shown in Table 4. Propylthiouracil seemed to be somewhat more effective in producing sustained remissions than thiouracil. In 6 of the 8 patients showing preliminary relapse before ultimate remission, such relapse occurred following thiouracil. Five of these six patients ultimately had a sustained remission after receiving propylthiouracil.

Previous reports (2, 3, 4, 5) suggest that sustained remissions following

TABLE 4. RELATION OF END-RESULTS TO TYPE OF DRUG USED

Antithyroid drug used	(a) Remission without relapse	(b) Remission after relapse	(c) Failures	Number of patients treated
Thiouracil	15	1	3	19
Propylthiouracil	15	2	2	19
Thiouracil followed by propylthiouracil	0	5	1	6
Aminothiazole	2	0	0	2
	32	*8*	*6*	*46*

the withdrawal of therapy with thiouracil derivatives may be expected in about 50 to 60 per cent of all thyrotoxic patients. They also suggest that the optimal period of treatment for the purpose of producing such sustained remissions should be about one year. The experience with the selected

group of patients here reported is not in accord with such suggestions, since in 82.6 per cent of our patients the disease has remained in remission for one year or longer following withdrawal of therapy. Furthermore, among the 38 patients showing a post-therapeutic remission for one year or longer, 21 received treatment for less than twelve months and 12 of these were treated for less than six months. Among 22 patients showing a post-therapeutic remission for two years or longer, 16 received treatment for less than one year and 10 of these were treated for less than six months.

SUMMARY

Long-term follow-up of 46 selected thyrotoxic patients after courses of thiouracil, propylthiouracil or aminothiazole, showed good results in 40 and failure in 6. Detailed comparison of the two groups revealed no criteria of prognostic value. Twenty-one patients showing good results received treatment for less than twelve months, and 16 of these for less than six months. Relapses following reduced dosage or withdrawal of therapy occurred in 8 patients who ultimately showed sustained remissions. Undesirable late effects of treatment were not observed.

ADDENDUM

Since this article was written, additional experience with the prolonged use of thiouracil compounds has been reported. Greenberg and Bruger (Observations on the prolonged medical management of toxic diffuse goiter with thiouracil and propylthiouracil, *Am. J. M. Sci.* **220:** 373 (Oct.) 1950) have reported remissions lasting six months or longer in 77 per cent of 39 selected patients with toxic diffuse goiter treated for twelve months with thiouracil or propylthiouracil. Iversen (Late results from continuous treatment of thyrotoxicosis with methylthiouracil, *J. Clin. Endocrinol.* **11:** 298 (March) 1951) has observed remissions of more than six months' duration in 78 per cent of 144 unselected thyrotoxic patients, following treatment for approximately one year with methylthiouracil.

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SERUM INORGANIC PHOSPHORUS IN THE DIAGNOSIS OF DIABETES MELLITUS

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THE oral or intravenous administration of glucose to normal individuals produces a temporary depression in the serum level of inorganic phosphorus. It has been suggested (1, 2) that the measurement of this phosphorus fall during the performance of glucose tolerance tests may be a valuable adjunct in the discovery of cases of early, latent, or so-called pre-diabetes mellitus. The importance of recognizing diabetes early in its course has received recent emphasis and any laboratory refinement which may facilitate its diagnosis requires careful evaluation.

METHODS

Serum inorganic phosphorus was determined during the performance of intravenous glucose tolerance tests in 28 normal controls who were internes, residents, medical students or student nurses and in 24 patients with diabetes mellitus selected from the outpatient diabetic clinic. Fourteen of the latter group were extremely mild diabetics, *i.e.*, they had not required insulin within the past five years, their fasting blood sugars ranged from normal to 175 mg./100 cc. and their 24-hour urine specimens were usually free of reducing substances. Most of them exhibited hyperglycemia and glycosuria only during periods of stress. Ten were classified as moderately severe diabetics because they had always required insulin, their fasting blood sugars were frequently elevated and glycosuria was often present. No special preparatory diet was prescribed prior to the test but it was ascertained that the previous diet was adequate. Tests were performed in the morning after an overnight fast. One-third gram of dextrose per kilogram of body weight in a 50 per cent aqueous solution was injected intravenously within 3 to 5 minutes and 0.1-cc. capillary blood specimens were obtained 15, 30, 60, 75, 90 and 120 minutes thereafter (3). True blood sugar was determined by the Lauber-Mattice method (4) and read on a Klett-Summerson photoelectric colorimeter. Duplicate specimens showed an average error of $\pm$ 1.4 mg. and a range of 0–6 mg. Serum inorganic phosphorus was determined before, and one and two hours after the glucose injection (5). In previous studies we ascertained that the maximum phosphate fall would occur within this time interval. Blood specimens showing any gross he-

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molysis were discarded. In 10 normal subjects phosphate was similarly measured after the intravenous injection of 50 cc. of normal saline solution In an additional 15 normal subjects serum inorganic phosphorus was measured before and 15, 30 and 60 minutes after the intravenous injection of Lilly's crystalline insulin in doses varying from 0.025 to 0.1 unit per kilogram of body weight.

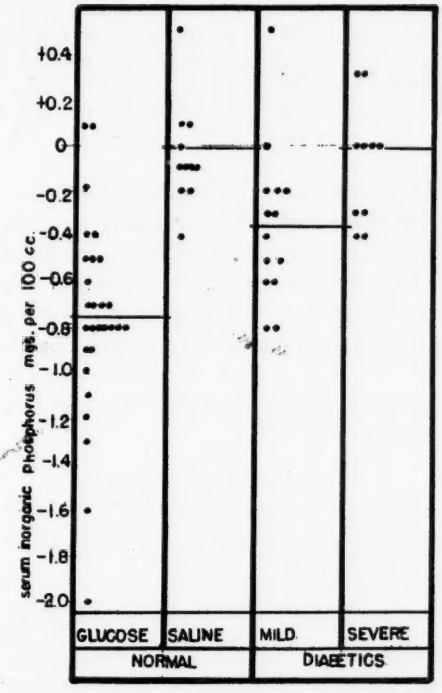

FIG. 1. Maximum change in serum inorganic phosphorus after intravenous glucose in normal subjects and diabetics, and after intravenous normal saline in normal subjects. Horizontal lines show the mean for each group.

RESULTS

In Figure 1 the maximum serum inorganic phosphorus change for the 62 subjects studied is shown. Table 1 outlines the mean phosphorus fall for the four groups. There was a statistically significant difference between the mild diabetic group and the normal controls (*i.e.*, $p < 0.01$). Eight of the 14 mild diabetic subjects but only 5 of the 28 normal subjects showed a phosphate fall of 0.4 mg. or less. The standard deviations of the normal and mild diabetic groups were substantial, however, and the degree of overlap

TABLE 1. THE MEAN FALL IN SERUM INORGANIC PHOSPHORUS AFTER INTRAVENOUS GLUCOSE

	Number of cases	Serum inorganic phosphorus, mg./100 cc.		
		Mean fall	Range	Standard deviation
Normal	28	0.76	0.1 to −2.0	±0.44
Mild diabetics	14	0.35	0.5 to −0.8	±0.34
Moderately severe diabetics	10	0.08	0.3 to −0.4	±0.26
Saline controls	10	0.04	0.5 to −0.4	±0.24

between the two groups was large, as depicted in Figure 1. The phosphate fall in normal subjects given saline intravenously was almost identical with the fall in the severe diabetic group given glucose. Figure 2 shows that the mean sugar curve of the mild diabetic group did not differ markedly from the normal controls, illustrating the type of diabetic subject selected for this study, *i.e.*, subjects with equivocal or mild depressions in dextrose tolerance.

In Table 2 an attempt to relate the degree of phosphate fall to the blood

TABLE 2. THE $\Delta G/\Delta P$ RATIO

	Mean	Range	Standard deviation
Normal	14.1	−100 to 170	± 43
Mild diabetics	182.4	− 4 to 940	±230
Moderately severe diabetics	579.3	60 to 960	±257

sugar value is shown using a modification of the $\Delta G/\Delta P$ ratio of DeVenanzi (6). ΔG represents the difference between the fasting and the 60-minute blood sugar, and ΔP the maximum diminution of serum inorganic phosphorus. Positive values were obtained when the 60-minute blood sugar was higher than the initial fasting value, and negative values when it was below the fasting level. There was a statistically significant difference ($p < 0.01$) between the means of both diabetic groups and the normals though here, too, the standard deviation and the degree of overlap were large.

In Table 3 the mean phosphorus fall after the intravenous injection of small doses of insulin is shown. When 0.1 unit per kilogram was adminis-

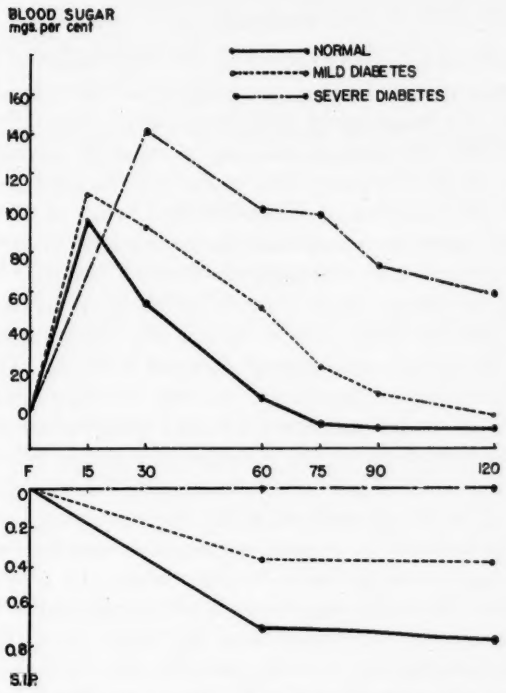

FIG. 2. The mean intravenous glucose tolerance curves and phosphorus fall (S.I.P.). Blood sugars and phosphorus values represent the milligrams change from the fasting value.

tered the phosphate fall was similar to that found in normal subjects given glucose intravenously. In order to produce a degree of hypophosphatemia similar to that noted in the mild diabetic group, less than 0.038 unit but more than 0.025 unit per kilogram would be required.

TABLE 3. THE FALL IN SERUM INORGANIC PHOSPHORUS AFTER THE INTRAVENOUS INJECTION OF INSULIN

Insulin dose (units per kilo body weight)	Mean fall in serum inorganic phosphorus, in mg.	Mean fall in blood sugar, in mg. per 100 cc.
0.1	0.85	58
0.075	0.55	44
0.05	0.44	21
0.038	0.43	22
0.025	0.15	16

DISCUSSION

The data presented demonstrate that the determination of serum inorganic phosphorus during the intravenous glucose tolerance test is of value as an adjunct in the diagnosis of diabetes mellitus. The difference in phosphate fall between the normal and the moderately severe diabetic was clear cut. The difference between the normal and the mild diabetic patients, though statistically significant, showed a wide range of overlap and diagnostic decisions based on phosphate determinations must be made with caution in this group. Only a phosphorus drop of 1.03 mg.[1] or greater definitely excludes the diagnosis of diabetes mellitus. When normal subjects were given as little as 0.038 unit of insulin per kilogram of body weight intravenously the serum phosphorus dropped 0.43 mg. The serum level of inorganic phosphorus appears to be very sensitive to insulin and it seems possible that many patients with mild diabetes are capable of producing sufficient insulin under the stimulus of injected glucose to provoke a substantial degree of hypophosphatemia. The mechanism of the phosphate fall is not clearly understood at the present time. It may be due to insulin secretion induced by hyperglycemia or it may be caused solely by the peripheral uptake of glucose (7). Calculating the $\Delta G/\Delta P$ ratio in an attempt to relate the blood sugar values to the phosphate fall did not increase the accuracy of the phosphorus test since here, too, the overlap between normal controls and diabetic patients was substantial.

Our results confirm the report of Forsham and Thorn (1) attesting to the value of measuring serum inorganic phosphorus as an aid to the diagnosis of diabetes mellitus. Whether measuring phosphorus changes during the oral glucose tolerance test will prove valuable has not been determined. McCullagh and Van Alstine (8), because they found normal phosphate curves during the oral glucose tolerance test in 56 per cent of 32 diabetic patients, felt that phosphate determinations were of little diagnostic value.

SUMMARY

The value of determining the serum inorganic phosphorus during the intravenous glucose tolerance test in the diagnosis of diabetes mellitus was examined. Studies in 28 normal controls and in 24 patients with diabetes mellitus demonstrated that phosphate measurements are of aid in diagnosis, though a significant degree of overlap between the two groups was noted.

The phosphate response to intravenous insulin was compared to the response to the intravenous injection of glucose. The results suggested that the mild diabetic was capable of secreting insulin and/or utilizing

[1] Twice the standard deviation of the mean of the mild diabetic group.

glucose, thus partly explaining the substantial overlap of the phosphate fall in normal subjects and in patients with mild diabetes mellitus.

Acknowledgment

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SEX-LINKED JUVENILE DIABETES MELLITUS

A GENETIC AND CLINICAL STUDY OF A FAMILY*

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THIS study presents data which suggest that genetic factors influence the occurrence of diabetes mellitus. In the family to be discussed, these factors are emphasized by the number of members affected in the immediate family and the occurrence of the disease in members of the same sex.

The family consists of 8 persons: father (F. T.—aged 39), mother (M. T. —aged 35), 3 boys (N. T.—aged 16, J. T.—aged 11, L. T.—aged 8), and 3 girls (L. T.—aged 14, C. T.—aged 12, and A. T.—aged 4). The father and the 3 girls are of average height and have brown hair and eyes; the mother and the 3 boys have blond hair and blue eyes. The growth curves of the boys and their mother fall within the lowest 1 per cent of the normal range. Mathematical analysis suggests that the growth rate and hair pigment are inherited in the same chromosome and that the growth retardation and blondness are inherited as recessive autosomal characteristics with a homozygous mother and a heterozygous father. The 3 boys have diabetes mellitus. The other members of the family show no glucose intolerance and, indeed, the females show a tendency toward a flat type of curve (Figs. 1 and 2). The family history was traced back for three generations on both the paternal and maternal sides. On the paternal side a history of diabetes mellitus was found in a great-aunt and in one of her daughters. No history of diabetes mellitus was found on the maternal side.

Using the genetic formula:

$$20\times(3/4)^3\times(1/4)^3=\tfrac{540}{4096}=\tfrac{1}{76}(a^6+6a^5b+15a^4b^2+20a^3b^3+15a^2b^4+6ab^5+b^6)$$

the chances are in favor of the diabetes mellitus being a recessive sex-linked characteristic with heterozygous parents (1/8) rather than a recessive autosomal characteristic with heterozygous parents using the genetic formula:

$$20\times(1/8)^3\times(3/8)^3=\tfrac{540}{262124}=1/485.$$

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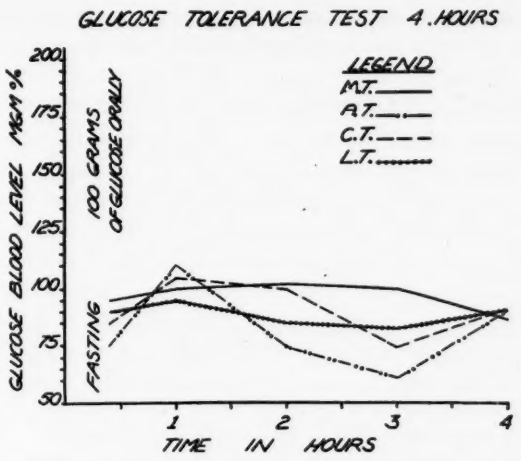

FIG. 1. Glucose tolerance in the mother (M.T.) and the sisters (A.T., C.T., L.T.) of the diabetic brothers.

The 3 patients with diabetes manifested their disease clinically at an early age: N. T.—age 5; J. T.—age 6; L. T.—age 4. The 2 youngest boys entered the hospital in impending diabetic coma and it was at this time that the disease was first discovered. These boys have since been well controlled on insulin and diet. The oldest boy first entered the hospital in diabetic coma at the age of 12, seven years after the disease was first diagnosed. His has been a rather difficult case to control, for he has manifested both clinical symptoms and chemical abnormalities characteristic of severe diabetic acidosis on two subsequent occasions.

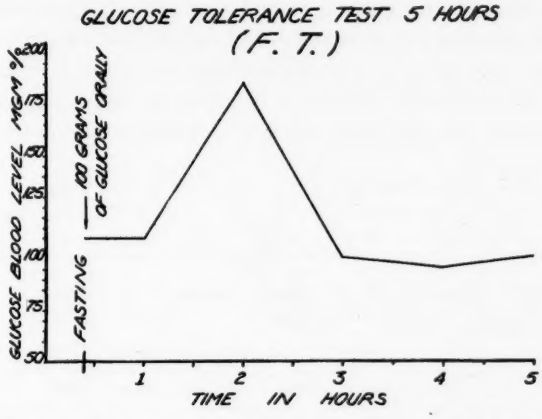

FIG. 2. Glucose tolerance in the father (F.T.) of the diabetic brothers.

TABLE 1. CLINICAL AND LABORATORY STUDIES OF THE DIABETIC BROTHERS

	N. T.	J. T.	L. T.
Age—(years)	16	10	8
Bone age (years)	13.3	9	$7\frac{1}{4}$
Weight (pounds)	95	65	54
Height (inches)	$54\frac{3}{4}$	51	$44\frac{3}{4}$
Chest film	N*	N	N
Sella turcica film	N	N	N
Complete blood count	N	N	N
Eosinophils (per cent of W.B.C.)	2	2	2
Protein-bound blood iodine (gamma/100 cc.)	4.4	5.0	5.1
Serum calcium (mg./100 cc.)	9.3	10.0	9.4
Serum phosphorus (mg./100 cc.)	4.8	5.0	4.4
Serum alkaline phosphatase (units/100 cc.)	8.8	7.8	8.8
Serum cholesterol (mg./100 cc.)	156	171	188
Serum albumin (Gm./100 cc.)	4.8	5.0	5.1
Serum globulin (Gm./100 cc.)	2.1	2.2	2.0
Thymol turbidity (units/100 cc.)	1	2	4
Cephalin flocculation (48 hrs.)	4+	4+	1+
Icteric index (units)	8	8	9
Urine analysis (except glycosuria)	N	N	N
17-Ketosteroid excretion (mg./24 hrs.)	12	4	3
Urine gonadotropin excretion (day)	N	N	N
Insulin requirements (units/day)	20 PZI,	10 PZI, 11 CZI	15 U PZI
Bromsulfthalein retention (5 mg./Kg.)	N	N	N

* (N = Normal)

It has been repeatedly noted (2–5) that most children with diabetes mellitus show little or no growth retardation if their disease is discovered early in childhood and is adequately controlled. Aside from patient N. T., whose diabetes has been difficult to control, the other 2 siblings have responded well to treatment. They are retarded in their growth. Because of this observation it was felt that a study should be made of the metabolic

TABLE 2. GENETIC CHARACTERISTICS OF THE FAMILY

	F. T.	M. T.	L. T.	C. T.	A. T.	J. T.	L. T.	N. T.
Taste of PTC (phenylthiocarbamide)	Bitter	Bitter	Bitter	No taste	?	Bitter	?	No taste
Isihara test for color blindness		Normal			Normal	Normal	Normal	Blind
Color of eyes	Brown	Blue	Blue	Brown	Brown	Blue	Blue	Brown
Tongue twisting ability	Yes	Yes	Yes	Yes	Yes	Yes	Yes	0

characteristics of each of the patients. A tabulation of the results appears in Table 1. There was no evidence of pituitary, adrenal, thyroid or gonadal disturbances. Results of hepatic function tests fell within normal limits except for the cephalin flocculation, which was 4+ in forty-eight hours in N. T. and J. T., and normal in A. T. Bromsulfthalein tests were made as follow-ups in N. T. and J. T., and the findings were normal.

Genetic characteristics other than diabetes mellitus are listed in Table 2.

No definite genetic formulation can be made from these data. It should be noted that patient N. T. has bilateral optic nerve degeneration of unknown etiology and idiopathic grand mal epilepsy. The latter appears to be unrelated to hypoglycemia, as determined by a history of at least several convulsions soon after meals, and as supported in part by the results of a six-hour insulin tolerance test.

SUMMARY

A genetic and clinical study is presented of a family of 8 persons (3 boys, 3 girls, and their parents) in which diabetes mellitus is present in the 3 male siblings. The diabetes mellitus is probably inherited as a sex-linked recessive characteristic with heterozygous parents. Other genetic characteristics are presented.

Considered mathematically, growth retardation and blondness in the same siblings are thought to be inherited as recessive autosomal characteristics with a homozygous mother and a heteroyzgous father.

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SPERMATOGENESIS AND APPARENT FERTILITY IN A EUNUCHOID MALE IN THE ELEVENTH YEAR OF ANDROGEN THERAPY

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THE purpose of this paper is to report several unusual occurrences in a male eunuchoid, at present in his twelfth year of androgen therapy. This patient is discussed for three reasons: the time intervals involved; the eventual achievement of normal testis size and histologic structure; and the presumptive demonstration of fertility and parenthood. A summary of the findings in this patient follows in the text.

Plum (1) in 1943 reported the production of spermatogenesis in a eunuchoid male, as evidenced by semen examination. He treated this patient for four years with chorionic gonadotropin, pregnant mare's serum hormone, and testosterone propionate. Heller and coworkers (2) shortly thereafter used chorionic gonadotropin supplemented by anterior hypophyseal gonadotropin for brief periods and found evidence of spermatogenesis in the testicular biopsy specimen.

Kinsell (3) reported in 1948 the appearance of spermatogenesis in a male with panhypopituitarism who was treated with testosterone pellets, and whose sperm count reached 18 million/cc. This work was followed shortly by a report (4) that spermatogenesis had been achieved by a eunuchoid male, given methyltestosterone unsuccessfully and then chorionic gonadotropin. The sperm count was 12 million/cc. Finally, 5 patients were recorded from the Lahey Clinic (5), 4 with eunuchoidism and 1 with a panhypopituitary problem. Treatment was by the administration of androgen to the eunuchoid patients, and chorionic gonadotropin and methyltestosterone to the other patient. Three of the 5 were said to have had children, although some of the evidence is open to criticism. Neither sperm count nor testicular biopsy was performed in the first instance; and the sperm count and testicular biopsy carried out shortly after the birth of the baby in the third case failed to show evidence of mature spermatozoa formation. The duration of treatment in these patients ranged from less than one year up to six years.

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CASE HISTORY

A. L., *U.H.* #567231, when first seen at the Presbyterian Hospital in December 1938, was 23 years old. He had been referred because of failure of chorionic gonadotropin to produce a significant effect during a six-month trial period in another institution. The history was irrelevant except for mumps without orchitis as a child.

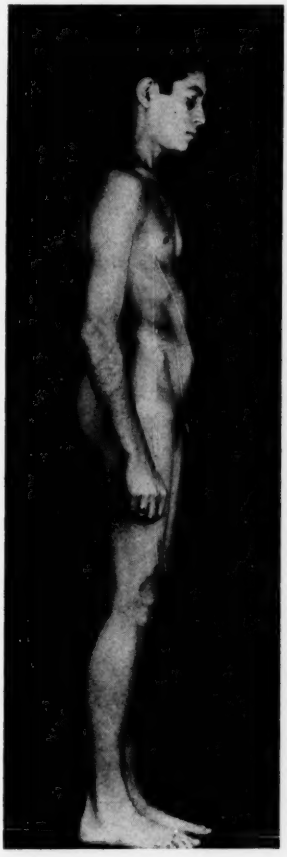

FIG. 1. Patient A.L., at age 23, prior to androgen therapy but following six months of chorionic gonadotropin administration elsewhere.

On physical examination, the patient showed the typical characteristics of eunuchoidism. He was 5 ft. 4 in. tall and weighed 110 pounds. He looked no older than 15 years of age. Front-view photographs prior to therapy and after seven years of androgen replacement have been presented elsewhere (6) and the lateral views are printed here (Figs. 1 and 2). The results of general examination were negative. The testes were infantile, each measuring about 0.5×0.5 cm., and of poor texture on palpation. The ex-

ternal genitalia were slightly developed, pubic and axillary hair was scant, and the prostate was not palpable. There was a slight fuzz on the upper lip.

Laboratory data. Urinary excretion values for total neutral 17-ketosteroids, estrogens and gonadotropins obtained initially and at the end of the therapy are presented in Table 1. The low gonadotropin excretion titer corresponds to that found in "Group A" of Howard and coworkers (7). Other laboratory studies gave essentially negative findings

FIG. 2. Patient A.L., at age 30, after seven years of androgen therapy.

and included X-ray examination of the sella turcica, oral glucose tolerance test, fasting serum cholesterol and basal metabolic rate determinations. Roentgenograms showed considerable delay in fusion of the various epiphyses of the crest of the ileum, femur, knee, elbow and wrist.

In December 1938, after a year of experimentation with various preparations and dosages of testosterone propionate in oil, a maintenance regimen was begun, with a dose of 25 mg. three times a week given intramuscularly. By November 1940 he had grown

TABLE 1. HORMONAL EXCRETION IN URINE BEFORE AND AFTER METHYLTESTOSTERONE ADMINISTRATION

Duration of treatment	Date	Total neutral 17-ketosteroids, mg./24 hrs.	Estrogens, R.U./24 hrs.	Gonadotropins, M.U./24 hrs.
None	1/ 4/39			36
6 mos.	7/12/39	8.6		10
1 year	1/ 8/40	8.2	5	10
1 yr. 1 mo.	2/ 5/40			30
1 yr. 10 mos.	11/ 4/40	10.5	5	10
	11/26/40	9.2	5	20
12 yrs.	9/12/50	7.9		>10 and <80

about 2 inches and had gained 23 pounds. Roentgenograms of the epiphyses remained essentially as before, except for advance in fusion of the epiphyses of knee and pelvis. The testes each measured about 1×1 cm. and the prostate was palpable but small. Secondary sex characters were reasonably well developed and of adult proportions. Libido and emissions had been present since shortly after the beginning of therapy.

The patient appeared well enough to be drafted into the army in April 1941, in spite of protest. All treatment thus abruptly ended. Three months later he collapsed while on an enforced march and was discharged from the service. He reappeared at the clinic in September 1941, with constant pain in his knees, legs and feet and a sense of muscle weakness, present since his physical breakdown in the army. Sharp regression in libido, frequency of erections and frequency of shaving were noted, as well as a loss of ejaculate. Examination revealed several changes. The patient was quite gray. Each testis was now about 1.5×1×0.5 cm. in size. X-ray examination of the epiphyses showed marked acceleration of development. An orthopedic consultation report stated that the pain in the legs was due to organic muscle weakness. This was confirmed by lack of response of the symptom to sedatives as placebos, and its dramatic disappearance on institution

TABLE 2. SPERM COUNTS DURING METHYLTESTOSTERONE TREATMENT

Date	Duration of treatment	Sperm count			
		Vol., cc.	Total, million/cc.	Per cent motile forms	Per cent normal forms
11/30/48	10 yrs.	2.5	11	50	
12/31/48		3.5	6	50	77
3/14/49		2.5	0*		
5/13/49			0		
7/14/49		3.0	6		
10/ 4/49		3.0	13**	80	69

* Bilateral testicular biopsy, 1/7/49.

** Wife pregnant 2½ months.

of methyltestosterone therapy in May 1942, in a dose of 50 mg. daily. The weakness and pain have not recurred since.

The ingestion of methyltestosterone became irregular in 1943 and 1944 and varied between 0.01 and 0.03 Gm. daily. By May 1945, the patient appeared an adult male in every way and looked older, rather than younger, than his chronologic age (Fig. 2). He now was 5 ft. $6\frac{3}{4}$ in. tall and 155 pounds in weight, a 3-inch and 45-pound gain from the start of treatment. Examination showed a normal sized prostate and external

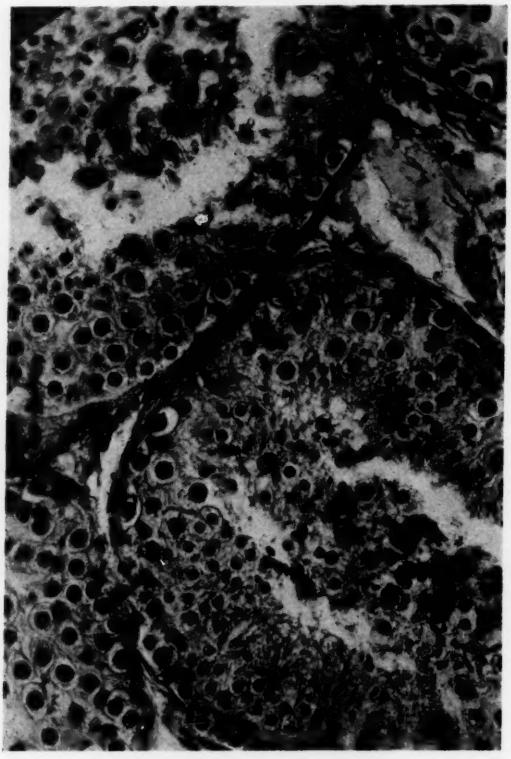

FIG. 3. Histologic section from biopsy specimen of right testis, showing achievement of relatively normal testicular structure during eleventh year of androgen therapy (Patient A.L.) ×600.

genitalia. Each testis still approximated 1.5×1×0.5 cm. His daily maintenance dose of methyltestosterone was now 0.01 Gm. three times daily. By June 1946, he had had an affair with the opposite sex. In December 1947, he announced his intentions of being married but stated that some difficulty was present in maintaining an erection during intercourse. This complaint was corrected with doubling the dose of methyltestosterone.

In June 1948, the patient was married and had himself decreased the dose of methyltestosterone to 0.01 Gm. three times daily again, without trouble. In November 1948 he returned for a routine check-up. He remarked that his testes had increased in size.

On examination, they had changed from the 1.5×1.0×0.5 cm. measurements hitherto recorded to about 2.5×2.0×2.0 cm., the left being slightly smaller. A semen specimen on November 30, 1948 revealed a volume of 2.5 cc., and 11 million sperm per cc., of which 50 per cent were motile forms (Table 2). The examination was repeated four weeks later, and revealed a count of 6 million sperm per cc., and 50 per cent motile forms with 77 per cent showing normal morphology.

A biopsy of the testis (Figs. 3 and 4) was consented to and performed in January

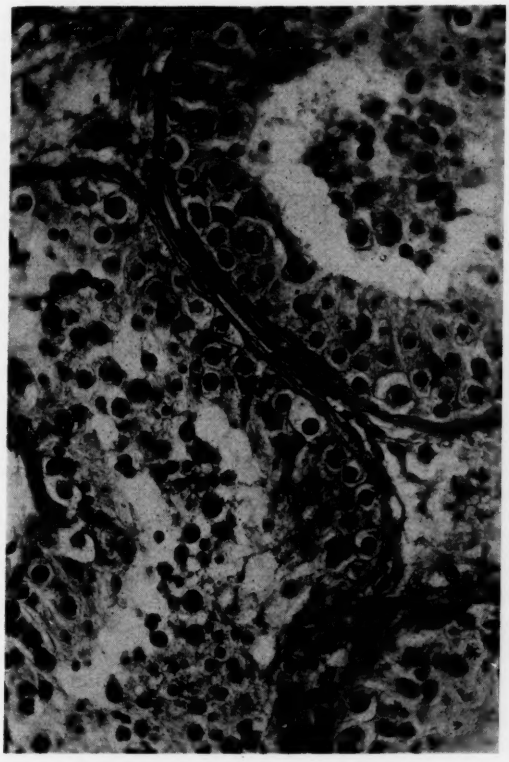

FIG. 4. Histologic section from biopsy specimen of left testis, showing persistence of testicular immaturity during eleventh year of androgen therapy (Patient A.L.) ×600.

1949 by Dr. J. S. Lattimer of the Department of Urology. The detailed histologic report by Dr. E. T. Engle, Department of Anatomy, follows:

"*Right testis*: Tubules unequal in size, uniform in contour. A few tubules fibrosed; majority with normal basement membrane and tunica propria. All stages of spermatogenesis present. Abundance of spermatozoa. Some tubules contain numerous early stages free in lumen—premature dehiscence. Some mitotic abnormalities with giant primary spermatocytes.

Interstitial cells few—tissue consists of fibroblasts.

Left testis: Tubules quite unequal in size. Some fibrosis. All tubules have greatly reduced cell population. All states of spermatogenesis including spermatozoa are present in some tubules but in greatly reduced numbers.

Interstitial cells sparse. Numerous intertubular fibroblasts.

Diagnosis: *Right testis*, approximately normal tubules. *Left testis*, reduced rate of spermatogenesis of immature testis."

July 18, 1949, the Urology Clinic added alpha-tocopherol to the regimen, 0.05 Gm. daily. October 4, 1949, the patient declared his wife to be two months pregnant. Fertilization occurred one and a half years after their marriage and about a month after the alpha-tocopherol therapy was instituted. A semen examination in October 1949 showed 13 million sperm/cc. with a volume of 3 cc. There were 80 per cent motile forms and 79 per cent normal forms at $1\frac{3}{4}$ hours. His wife was delivered May 15, 1950, of a normal

TABLE 3. TABLE SHOWING BLOOD GROUPS OF PATIENT, MOTHER AND SIBLING

Patient, blood groupings* $A\text{-}1_M$			Sibling, blood groupings A_{MN}			Mother, blood groupings O_N		
Rh		+**	Rh		+**	Rh		+†
Anti-Rh^0	(D)	+	Anti-Rh^0	(D)	+	Anti-Rh^0	(D)	+
Anti-Rh^1	(C)	+	Anti-Rh^1	(C)	+	Anti-Rh^1	(C)	+
Anti-Rh^{11}	(E)	−	Anti-Rh^{11}	(E)	−	Anti-Rh^{11}	(E)	+
Anti-Rh^1	(C)	−	Anti-Rh^1	(C)	−	Anti-Rh^1	(C)	+

* The author wishes to thank Dr. John Scudder for the grouping of the blood.

** Baby and father—Rh^1, homozygous presumably.

† Mother—Rh^1, Rh^2 presumably.

male child. Blood obtained from the offspring was secured for detailed blood grouping and compared with the blood groupings from the patient and from the mother (Table 3). No blood groups were present which were inconsistent with the patient being the actual parent of the child.

COMMENT

It is unfortunate that no sperm count was obtained before the doubling of testis size which occurred in the nine-month period following his marriage and in the tenth year of his androgen therapy. It seems reasonable to presume that the change in testicular size may have been associated with a corresponding acceleration of spermatogenesis, especially since biopsy showed that one of the two testes had retained some features of its immature structure.

The evidence for fertility despite the relatively low sperm count seems good. McLeod (8) has presented evidence that males whose fertility has never been questioned may have oligozoospermia of the degree shown by the present patient. Moreover, the blood groups of patient and offspring

are not incompatible with the presumption that the patient is the true father.

Of interest is the 10-year lag before a change in testis size took place. Several possible mechanisms seem available to have produced this phenomenon. It is conceivable that the sexual stimulation of married existence may have induced increased gonadotropin secretion by the anterior pituitary, apart from any effect from methyltestosterone. This would fit with the growing body of evidence that the anterior pituitary may in turn be influenced by the central nervous system, particularly the hypothalamus. Against such a view is the fact that the evidence for gonadotropin release by this means, although clear in the rabbit, has not been extended to man. It is of interest that Plum's patient also married, after two and a half years of therapy, and within the next two years had achieved normal testis size and a sperm count of 1.5 million per cc. This was attributed to an increased dosage of chorionic gonadotropin during the fourth year of treatment.

Another possibility is that the anterior pituitary may have been stimulated by continued testosterone therapy to release gonadotropin, assuming initial failure of secretion to be responsible for the eunuchoidism. Evidence of primary failure of gonadotropin release is weak in the patient here under discussion, and depends on the fact that no excess gonadotropin was demonstrated in the urine assays conducted before and during the first two years of therapy. It is odd that secretory activity should have been induced, since suppression of gonadotropic secretion by the anterior hypophysis is the usual response to the administration of relatively large doses of methyltestosterone; inhibition of the testis and azoospermia have been repeatedly noted in short-term experiments in normal subjects. However, the results of long-term experiments of this nature, recently described, indicate that spermatogenesis may be resumed despite the initial suppression (9, 10), when androgen therapy is discontinued. It appears in our patient that the anterior pituitary may have escaped from the suppressive effect of androgens in the face of continued administration. This view is consistent with the repeated presence of low-normal amounts of urinary gonadotropin after the second year of treatment.

A third possibility is that the effect of testosterone in this patient was directly upon the testis tubules and is unrelated to gonadotropin release.

A final possibility is that the sequence of events was spontaneous and unrelated to the events described above. This seems least likely.

The inadvertent institution of alpha-tocopherol therapy a few days prior to fertilization appears to be pure coincidence and is hard to envisage as playing a role in the subsequent outcome, both in view of the short time

interval here, and the general unsuccessful experience in treating sterility with this agent.

SUMMARY

The evidence for the achievement of spermatogenesis and fertility in a eunuchoid male in the tenth and eleventh years of treatment with androgens is detailed. The results of semen counts, testicular biopsy and blood groupings are presented.

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INHIBITION OF A CERVICAL THYROID GLAND BY A FUNCTIONING STRUMA OVARII

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THE purpose of this report is to present evidence suggesting that the endogenous secretion products of a functioning struma ovarii may inhibit the function of a normal cervical thyroid. In the past the inhibition of the normal thyroid by the ingestion of thyroid substance has been measured by using only one of the following assays of thyroid function: the basal metabolic rate (1), the level of serum protein-bound iodine (2), or the ability of the thyroid to concentrate radioactive iodine (3). In this investigation, all three methods of assaying thyroid activity were utilized in the study of a patient after the removal of a struma ovarii.

CASE REPORT

K.F., a 48-year-old white housewife, was treated in January 1950, for an attack of acute pyelitis. During this illness she noted the onset of palpitation and her physician made a clinical diagnosis of auricular fibrillation. The cardiac irregularity disappeared coincident with the administration of digitalis and the subsidence of the renal infection. At this time a large pelvic mass was palpated. X-ray examination of the urinary tract by intravenous pyelography revealed a large calculus along the course of the right ureter, a right hydronephrosis, and no left kidney shadow.

About eighteen years before, the patient has been hospitalized in a Viennese Hospital. At that time, she had seen a note on her hospital record stating that she had a pelvic mass. She had not had any lower abdominal symptoms. Her menarche occurred at the age of 15. The menses were regular and normal. The patient spontaneously aborted a 3-month fetus twenty years previously and had one normal full-term delivery the following year.

There had been no weight loss in ten years despite a poor appetite. There was no preference for cold weather, no excessive sweating and no diarrhea.

On March 5, 1950, the patient was admitted to the Gynecological Service of the Maimonides Hospital of Brooklyn for the removal of the pelvic mass. *On physical examination* the patient appeared to be a thin, narrow-faced, poorly nourished female. She was apprehensive, over-active, and perspiring profusely. The pulse rate was 104 beats per minute. The blood pressure was 152/70. The eyes were prominent but there was neither stare nor proptosis. No lid lag was noted. The thyroid was not palpable. The heart was not enlarged, the rhythm was regular, and a faint systolic murmur was audible at the apex. Abdominal examination revealed no flank mass and no flank tender-

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ness. A large firm mass was felt in the suprapubic area which extended to two inches below the umbilicus. Vaginal examination revealed that the pelvis was filled with a hard mass, continuous with the one felt by the abdominal palpation. No other pelvic mass was felt. The extremities were neither tremulous nor edematous.

Laboratory data prior to the laparotomy included a normal blood count, 2 plus albuminuria and evidence of a mild pyuria. Two urine cultures showed Escherichia coli. The blood urea nitrogen was 18 mg. per cent and the serum cholesterol, 333 mg. per cent. The phenolsulfonphthalein excretion was 45 per cent in two hours.

The interne, Dr. Marvin H. Sentnor, suggested that this patient's pelvic tumor might be an ovarian cyst. Because he believed that the patient had hyperthyroidism, no palpable thyroid and an ovarian tumor, he suggested the possibility that this patient had a struma ovarii. Unfortunately, no metabolic or thyroid function studies were made prior to operation.

At operation, on March 5, 1950, a subtotal hysterectomy and a bilateral salpingo-oophorectomy were performed. The uterus exhibited a subseptus type of endometrial cavity and two small myomata. The left tube was retort-shaped and intimately bound to the ovary. No fimbriated end could be visualized but the distal portion of the tube was dilated to a diameter of 2.5 cm. Pressure on this portion of tube elicited a flow of hemorrhagic fluid into the left uterine cornu. The left ovary was slightly enlarged and contained a yellow body with corrugated borders measuring 0.6 cm. in diameter. The right tube was slightly dilated but patent. The right ovary consisted of a firm mass measuring 21.0×14.0×5.0 cm., surrounded by a smooth yellowish-gray capsule with mottled hemorrhagic areas. It cut with moderate and occasionally calcific resistance, to reveal a predominantly solid tan tissue interspersed with several cysts measuring up to 8.0 cm. in diameter. These cysts possessed ragged walls and contained up to 100 cc. of bloody fluid. One cyst, 5 cm. in diameter, possessed a smooth gray lining and was filled with cheesy, gray, hair-containing material.

Microscopic examination of the uterus revealed a resting endometrium. The left tube and ovary were involved by a mass of organized granulation tissue incorporating columnar-lined, blood-filled glands, and many hemosiderin-laden macrophages. A regressing corpus luteum was also present. The bulk of the right ovary consisted of masses of solid and patent acini lined by cuboidal cells. Inspissated colloid filled many of the acini. Large areas of hyalinized fibrous tissue were present in addition to a cyst lined by hyalinized fibrous tissue, foamy macrophages, foreign body giant cells, and occasional hair shafts.

The pathologic diagnoses were: 1. Dermoid cyst with struma ovarii of the right ovary. 2. Endometriosis of left tube and ovary. 3. Uterus subseptus. 4. Resting endometrium. 5. Small myomata uteri.

Thirty-six hours postoperatively, the patient's temperature rose to 101.8° F. and she appeared to be acutely ill. The possibility of incipient thyroid storm was considered. However, the absence of tachycardia, of recent weight loss, of preference for cold, of eye signs suggestive of hyperthyroidism, and of a palpable thyroid gland were considered strong evidence against thyrotoxicosis. The pulse rate was never above 100 per minute despite the mild fever. The temperature and pulse both fell to normal on the third postoperative day and remained normal throughout her hospital stay.

METABOLIC STUDIES

Methods

Two days after removal of the struma ovarii, serial determinations of the patient's serum protein-bound iodine level were begun. Five days

postoperatively, serial studies of radioactive iodine accumulation by the cervical thyroid, and the urinary excretion of radioactive iodine were undertaken. Beginning on the twelfth postoperative day, serial basal metabolic rates were determined.

The struma ovarii was kept in a deep-freeze unit. Parts were removed and chemically assayed for protein-bound iodine content.

Protein-bound iodine measurements of the serum and tissue were determined by the method of Barker (4). The normal serum values in this laboratory are from 3 to 8 gamma per cent. The accumulation of radioactive iodine by the thyroid gland was determined twenty-four hours after the oral ingestion of tracer doses of 25 microcuries of I^{131}. A shielded Geiger-Müller tube was placed 35 cm. from the neck. A one-inch-thick lead block was used to shield the thyroid area in order to determine the background count from the patient's body, excluding the thyroid area. The difference between these two readings was considered the thyroid accumulation. In this laboratory the normal thyroid accumulation at twenty-four hours is from 10 to 40 per cent of the administered dose. Twenty-four-hour urine specimens were collected on the first and second day after I^{131} administration. The radioactivity of the orally administered dose and of the urines was measured in a liquid counter, using Marinelli beakers. Skanse (5) has reported that the normal excretion of I^{131} during the first twenty-four hours varies from 40 to 81 per cent of the ingested dose; however, during the second twenty-four hours, the urinary excretion is always less than 12 per cent, in the normal person. The basal metabolic rates were measured on the Benedict-Roth machine.

Results

Protein bound iodine: Two days after removal of the struma ovarii, the serum protein-bound iodine level was 7.3 gamma per cent. Six days later it had fallen to 3.4 gamma per cent. On the twelfth postoperative day it was below the normal range (3 to 8 gamma per cent) at 2.5 gamma per cent. One week later it was still below normal, 2.1 gamma per cent. On the twenty-seventh day postoperatively, the protein-bound iodine began to rise and was 3.1 gamma per cent. The three determinations during the next month were all in the normal range, 3.5 to 5.5 gamma per cent.

Thyroid uptake and urinary excretion of I^{131}: Studies of the uptake of radioactive iodine by the thyroid were made on the fifth, twelfth, and twentieth days; all values were below 3 per cent (normal uptake is from 10 to 40 per cent). Because it was not deemed advisable to give too many tracer doses of I^{131}, the next study was done on the thirty-eighth postoperative day, when the rise in the protein-bound iodine of the twenty-seventh day had been reported. The uptake of I^{131} on the thirty-eighth postoperative day was 23 per cent. The cervical thyroid uptake of I^{131} was retested

two weeks later and was found to be 21 per cent of the administered dose.

The urinary excretion of I^{131} was studied on the fifth postoperative day. The excretion during the first twenty-four hours after ingestion of the tracer dose was 73 per cent; during the second 24-hour period there was an additional 17 per cent excretion. According to Skanse (5), this high urinary excretion during the second 24-hour period is very suggestive of the lack of functioning thyroid tissue. He reported the normal range of excretion during the second day to be between 0 and 12 per cent. All his myxedematous patients excreted more than 12 per cent during the second day.

TABLE 1. PROTEIN-BOUND IODINE CONTENT

	Gamma per gram
Struma ovarii	5.8 and 4.9
Normal ovary	0 and 0
Normal thyroid	177 and 187

Basal metabolic rates: The basal metabolic rates varied between −2 and +9 per cent. There was no correlation between the variations in the level of serum protein-bound iodine or the uptake of I^{131} by the thyroid on the one hand, and the basal metabolic rate on the other.

Chemical analysis of the struma ovarii: Frozen blocks of the struma ovarii of this patient, and normal thyroid and ovarian tissue of other patients were analyzed chemically for protein-bound iodine. Table 1 reveals that the struma ovarii did contain protein-bound iodine; however, its content was considerably less than that of normal thyroid tissue. The concentration of protein-bound iodine in the normal thyroid tissue of another patient, approximately 180 gamma per gram of frozen wet tissue, is within the range reported by Gutman *et al.* (6) in their chemical analyses of thyroid glands of normal individuals.

DISCUSSION

Emge (7) reported that only from 5 to 6 per cent of strumae ovarii are associated with clinical hyperthyroidism. Gusberg *et al.* (8) noted that 8 of 31 patients with this type of tumor were thyrotoxic. Removal of the ovarian tumor has been associated with disappearance of hyperthyroidism unless metastatic spread of the thyroid tissue had occurred. Chemical, pharmacologic, and biologic data have been presented by Plaut (9) to indicate that strumae ovarii contain thyroxin-like material.

Although these data prove that a struma ovarii can influence body metabolism, the literature reveals little to indicate that a struma ovarii

can influence the cervical thyroid gland. Trapl (10) reported that in his patient a moderate sized goiter and questionable hyperthyroidism developed five months after the removal of a struma ovarii. He stated that the appearance of the goiter and mild hyperthyroidism suggested that the removed struma ovarii had been functioning and that the cervical thyroid was stimulated after the removal of the ovarian tumor. Other than this

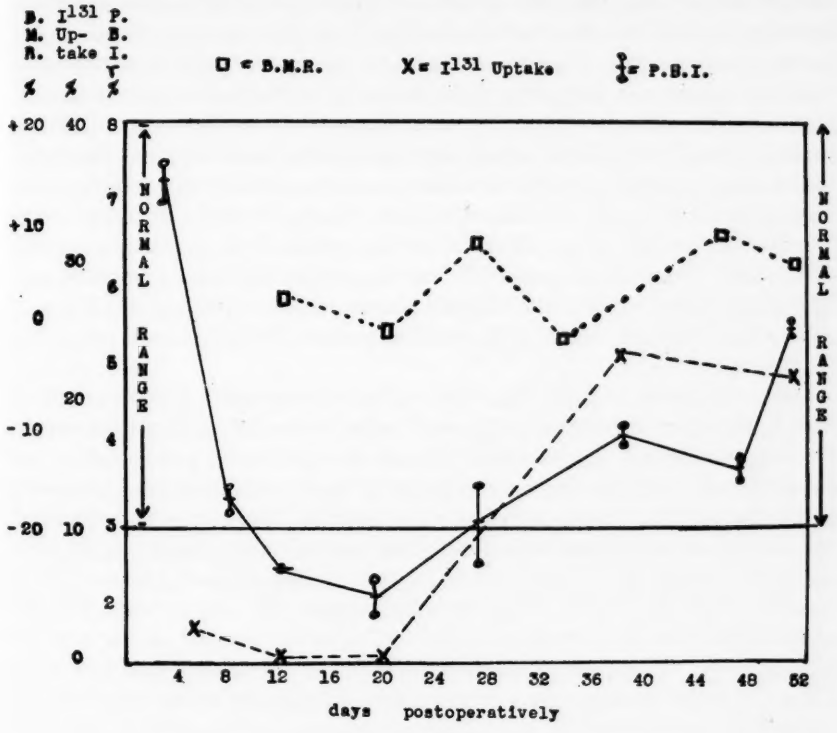

FIGURE 1

inconclusive data, no reported evidence was found to indicate that a functioning struma ovarii had inhibited a cervical thyroid gland.

Cope *et al.* (11) have demonstrated that the marked absorption of radioactive iodine by single adenomas, with or without clinical hyperthyroidism, can be associated with depression of the activity of the surrounding uninvolved thyroid gland. Farquharson (1), Winkler (2), and Greer (3) have presented data indicating that the ingestion of thyroid substance inhibits the activity of the normal thyroid gland. Unpublished data from this laboratory indicate that the ingestion of 2 grains of desiccated thyroid per day

decreases the thyroid uptake of I^{131} to hypothyroid levels without causing either symptoms or signs of hyperthyroidism, an increase in basal metabolic rate or an increase in the level of serum protein-bound iodine.

The availability of these two newer techniques, namely, the determination of the serum protein-bound iodine and of the uptake of I^{131} by the thyroid, made it possible to study subtle rapid changes in thyroid function of our patient after the removal of a struma ovarii. These changes in the status of thyroid function would not have been detected by studies of the basal metabolic rate. The following data favor the suggestion that this patient maintained her euthyroid status by endogenous secretion of a thyroxin-like substance from the struma ovarii: 1) the high normal serum protein-bound iodine level on the second postoperative day, 2) the rapid fall of the serum protein-bound iodine to a subnormal level by the twelfth postoperative day, 3) the failure of the cervical thyroid to concentrate I^{131} during the first three weeks after the removal of the struma ovarii, 4) the high urinary excretion of I^{131} on the second day after ingestion, and 5) the histologic evidence of thyroid tissue and the chemical evidence of functioning thyroid tissue in the ovarian tumor. These data are presented in Figure 1.

It is of interest to note that the level of the circulating protein-bound iodine fell below normal and remained below normal for at least one week. During this period, the cervical thyroid was dormant, as proved by an extremely low cervical thyroid uptake of I^{131} and the high urinary excretion of I^{131} during the second day after I^{131} ingestion. However, when the level of the circulating protein-bound iodine rose to the normal range, the cervical thyroid was then found to be functioning normally in its ability to concentrate I^{131}. These data strongly suggest that the cervical thyroid was inhibited by the secretory products of the struma ovarii, either directly or indirectly, via pituitary inhibition. Once the circulating protein-bound iodine of ovarian origin had decreased to subnormal levels, the cervical thyroid began to function normally and was presumably able to raise the circulating protein-bound iodine to normal levels.

These data indicate that this patient responded to the removal of the struma ovarii in a fashion similar to the response of normal individuals to the cessation of the oral administration of exogenous thyroid, as noted in the reports of Farquharson (1), Winkler (2) and Greer (3). The serum protein-bound iodine of Winkler's 2 patients was subnormal four weeks after the cessation of oral ingestion of 5 grains of desiccated thyroid. The level of the protein-bound iodine returned to normal in 1 of these 2 patients on the tenth week after the thyroid ingestion had stopped. In our patient, three weeks after the removal of the endogenous source of thyroid sub-

stance (the struma ovarii), the serum protein-bound iodine was below normal. On the thirty-eighth postoperative day, the serum protein-bound iodine level reached the normal range and persisted at this level. Thus our patient responded very much as did the 2 patients of Winkler.

Greer (3), using the radioactive iodine uptake as the assay of thyroid function, reported that the majority of his subjects' thyroid glands returned to normal function within two weeks after the cessation of thyroid ingestion. The thyroid glands of some of his subjects remained suppressed even at the end of six to nine weeks. The thyroid gland of our patient became active between the third and fifth week after removal of the suppressing organ, the struma ovarii.

Several of Greer's subjects had been taking thyroid substance for several years. The return of their thyroid glands to normal activity was as rapid as in those subjects who had taken thyroid for only a few weeks. Our patient had a pelvic mass for at least eighteen years. It is interesting to speculate that this dermoid tumor had depressed her cervical thyroid for a prolonged period of time. Despite this, within three to five weeks, the depressed thyroid had become normally active. These findings corroborate Greer's report.

The basal metabolic rate, long the main investigative tool of the student of thyroid function, failed to reflect the rapid changes in the status of thyroid activity. It is noteworthy that on the twentieth postoperative day, both the protein-bound iodine and the I^{131} uptake were in the myxedema range and yet the basal metabolic rate was normal (-1 per cent). Perhaps the storage of thyroxin or the stimulus of the body metabolism from thyroxin lasts for at least one week after the serum protein-bound iodine level has fallen to low values. This might explain the failure to find a low basal metabolic rate in this patient. It is certain that the subtle physiologic interrelationship between the level of the circulating thyroid hormone and the activity of the thyroid glandular tissue in this patient could not have been demonstrated if the basal metabolic rate were the only measure of thyroid activity.

It is interesting to speculate as to the mechanism by which the thyroid is inhibited by either endogenous or exogenous thyroid hormone. Kuschinsky (12), and later Purves (13), have demonstrated that orally administered thyroid decreases the thyroid-stimulating hormone of the rat's pituitary to less than 5 per cent of its normal content. This would suggest that high levels of circulating thyroid hormone inhibit thyrotropic hormone formation. On the other hand, Cortell and Rawson (14) have reported convincing evidence that, in the rat, thyroid hormone can inhibit the action of thyroid-stimulating hormone on its end organ. Studies are now in progress

in this laboratory to determine how the normal human thyroid, which has been depressed by exogenous thyroid hormone, responds to thyroid-stimulating hormone.

With the availability of the tracer radioactive iodine studies, it should be possible to make the diagnosis of a functioning struma ovarii by determining the I^{131} concentration over the pelvic tumor, preferably two to three days after the ingestion of the radioactive iodine and after the urinary bladder has been emptied. It is postulated that the findings of large concentrations of I^{131} over such a pelvic tumor would be useful in confirming the clinical impression of a struma ovarii. No report has been noted in the literature in which this test has been used to diagnose struma ovarii, although it has been of proved value in diagnosing functioning metastases of thyroid cancer.

SUMMARY

1. A case of dermoid cyst of the ovary with functioning thyroid tissue (struma ovarii) is presented. Serial studies of thyroid function were made after removal of the struma ovarii.

2. The serum protein-bound iodine fell from a high-normal concentration to subnormal levels in the first twelve days postoperatively, and then began to rise between the nineteenth and twenty-sixth day postoperatively, reaching normal levels on the thirty-eighth day.

3. The cervical thyroid uptake of I^{131} was markedly decreased up to the twentieth postoperative day but by the thirty-eighth postoperative day it was normal.

4. The basal metabolic rate remained within normal limits and did not vary significantly during this study.

5. The teratoma, histologically a struma ovarii, contained functioning thyroid tissue, as proved by chemical studies.

6. It is postulated that a functioning struma ovarii depressed the cervical thyroid, that the removal of the ovarian thyroid tissue resulted in a rapid decline of circulating protein-bound iodine, and that the period of low serum protein-bound iodine released the cervical thyroid from its depressed state.

Acknowledgment

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CHROMAFFIN CELL TUMOR SIMULATING MALIGNANT HYPERTENSION

REPORT OF A CASE WITH SURGICAL CURE

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ALTHOUGH every patient with a functioning chromaffin cell tumor probably has paroxysmal attacks of hypertension, a history of such attacks is not always readily obtainable (1), or their paroxysmal nature may be masked by the more chronic phase of the disease. It becomes necessary, therefore, to revise our concepts of the clinical picture, lest the correct diagnosis be missed in those cases which do not conform to the previously defined "paroxysmal hypertensive" syndrome.

Any or all of the effects of the "adrenal-sympathetic syndrome" may be found in these patients and are presumably due to the release of an excessive amount of epinephrine or epinephrine-like substance from the hormone-producing tumor. The many symptoms of these tumors are too numerous to list here (1–19), but may be found in the excellent reviews by Belt and Powell (2), Linder and Tyler (3), and Green (4). For clinical application, however, the diagnosis should be considered for the following groups of patients:

1. Normotensive patients with paroxysmal attacks of hypertension, headache, palpitation, epigastric or substernal distress, nausea and/or vomiting, sweating, pallor or flushing and angiospastic changes in the extremities.
2. Chronic hypertensive patients, especially when their hypertension is associated with a high basal metabolic rate, marked tachycardia, and hyperglycemia. This group may or may not have paroxysmal symptoms as a prominent feature; their symptoms often mimic those of malignant hypertension.
3. Normotensive or hypertensive patients in whom severe hypertension, pulmonary edema, shock, or sudden death occur without apparent cause; the occurrence of these symptoms during the course of anesthesia or an operative procedure, no matter how minor, is especially significant.

The symptoms characteristic of one group are, of course, not confined to the patients in that group; a particular patient may have any or all of the symptoms, regardless of group. A case is here presented which belongs primarily in group 2 but some of the symptoms are those ascribed to groups 1 and 3.

The purpose of this article is to describe a case of chromaffin cell tumor which simulated malignant hypertension and to discuss some of the difficulties in diagnosis and management.

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CASE REPORT

Mrs. G. C., a 22-year-old housewife, was admitted to the Mary Fletcher Hospital, Burlington, Vermont, on October 23, 1947, with a chief complaint of severe headache and blurring of vision. She stated that she had been well until the day following delivery of her last child, which occurred six and one-half months previously at another hospital. Following an uneventful pregnancy and normal delivery, nineteen hours post-partum she experienced a severe headache which lasted about half an hour. At this time her blood pressure was found to be 220/130. She was discharged on the fourth hospital day and no record of further blood pressure recordings taken during this time could be obtained. From that time on, she had recurrent headaches for which she visited several physicians, all of whom found her blood pressure to be elevated and advised hospitalization. The headaches were severe and incapacitating but between attacks she was well enough to help her husband in the hayfields. One week prior to admission she had her glasses changed by an optometrist and the following morning on arising she noted blurred vision in the right eye, which continued to the time of admission to the hospital. The vision in the left eye had been poor for many years and she noted no change during the present illness. On the day prior to admission she was seen by another physician who found the blood pressure to be 182/130 and referred her to an ophthalmologist for fundoscopic examination. He reported grade 4 hypertensive retinal changes and she was admitted to the hospital for study.

Family history: Her father died at 62 of hypertension. Her mother died at 31 of osteomyelitis or tuberculosis of the bone. Her mother, several siblings, and her three children had "liver spots" scattered all over their bodies.

Past medical history: The patient suffered an instrumental birth injury, causing ptosis of the left upper eyelid. At the age of 12 years, two "blood tumors" were removed from the left upper eyelid and she stated that the vision in that eye had been impaired since that time. She had an appendectomy at 17, and uneventful pregnancies at 18, 19, 20, and 22. Her third baby died at 8 days of age, from an undetermined cause. During the most recent pregnancy she stated that her only complaint had been backaches during the last trimester. Her husband however stated that she had had occasional attacks of palpitation, shortness of breath and a feeling of weakness during the past two years. At no time before the operation could this information be obtained from the patient. (Following the operation she did recall these episodes but considered them to be of little consequence, as they in no way were comparable to the incapacitating headaches experienced with the present illness.) She had three nose bleeds during the previous few months, the last one being three weeks prior to admission and brought on by exertion. Her maximum weight was 109 pounds three years ago. She weighed 95 pounds prior to her recent pregnancy and 86 pounds immediately after delivery. She had noted "liver spots" about the body, which she stated had been present since birth. A tender tumor mass on the right forearm had also been present since childhood and had not increased markedly in size.

Physical examination: The patient was a small thin blonde female. The blood pressure was 220/140 in both arms in the reclining position. The pulse was regular at 100. Multiple "cafe au lait" spots were scattered over the trunk; they were most prominent on the abdomen and back; there were a few on the thighs and upper arms. The left eyelid was thickened at its margin and most of the lashes were missing; there was a moderate ptosis and several surgical scars extended across the upper lid. The left pupil was slightly larger than the right and somewhat irregular in outline. Both pupils reacted to light but the left did not react to accommodation. There was a concomitant left convergent

strabismus of 20 degrees. The right eye could detect hand movements at 8 feet, the left eye at 10 feet. Ophthalmoscopic examination revealed the media of the right eye to be clear. There were numerous small superficial flame-shaped hemorrhages in and about the disc. The disc margins were indistinct. There were three diopters of papilledema. The retinal arteries were generally narrowed and the veins were dilated. There was a diffuse edema of the retina with numerous areas of "cotton wool exudate." The macula revealed a fully formed macular star and numerous small hemorrhages were seen adjacent to the retinal vessels, both above and below the disc. The retinal vessels were hidden in some areas by overlying edema. Examination of the left eye showed essentially

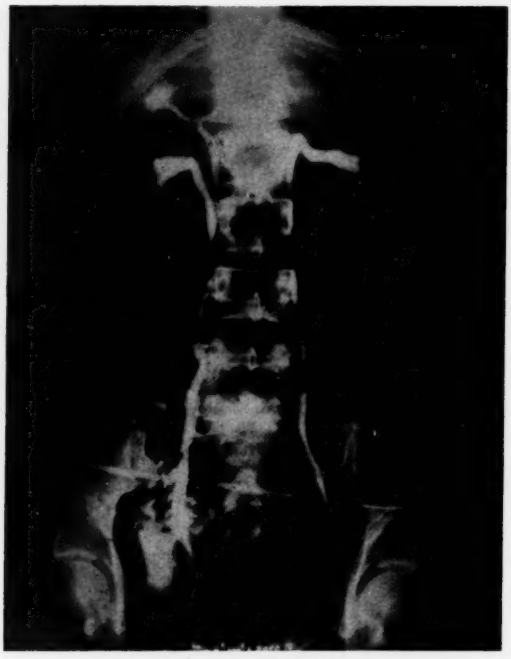


FIG. 1. Retrograde pyelogram showing double pelvis and ureters on the right, and a low single pelvis on the left.

the same condition, though more difficult to see, because of an irregular astigmatism of the cornea. The papilledema of the left optic disc was definite. The thyroid was not palpably enlarged. The lungs were clear and the heart was not enlarged. The aortic second sound was greater than the pulmonic second sound. The liver was palpably enlarged. The kidneys were not palpable and no masses could be felt in the renal areas. No edema of the extremities was noted. A walnut sized tender tumor was palpable on the right forearm about 2 cm. below the elbow. This mass was freely movable under the skin, and did not appear to be attached to the underlying muscles or tendons. The dorsalis pedis and posterior tibial pulsations were readily felt.

Laboratory examinations: Urinalysis showed a specific gravity of 1,028, four plus albumin, many pus cells with occasional blood cells and occasional finely granular casts.

A complete blood count was not remarkable. The serum protein was 6 Gm. per cent; nonprotein nitrogen, 44.8 mg. per cent, cholesterol, 395 mg. per cent; and potassium, 15.7 mg. per cent. A phenolsulphonphthalein test showed 60 per cent excretion of the dye in ten minutes. The basal metabolic rate was +51 per cent.

Nothing unusual was noted on x-ray examination of the chest; skull radiographs showed a slightly enlarged sella turcia with a slight dorsal angulation of the posterior clinoid processes. An intravenous pyelogram revealed apparently normal excretion of dye by the right kidney, which was normal in size and position. Dye excreted by the left kidney was slightly less distinct, although the kidney appeared to be functioning. There was a double pelvis and ureter on the right with a suggestion of a double ureter on the left, but this could not be definitely determined. There was a rapid passage of the dye to the bladder on both sides. Retrograde pyelograms (Fig. 1) confirmed the presence of a double pelvis and ureters on the right. The two ureters appeared to join a short distance above the bladder. There was no evidence of intrinsic disease or of pressure by an extrinsic mass on the right. On the left, a single pelvis was demonstrated which was situated considerably lower than usual.

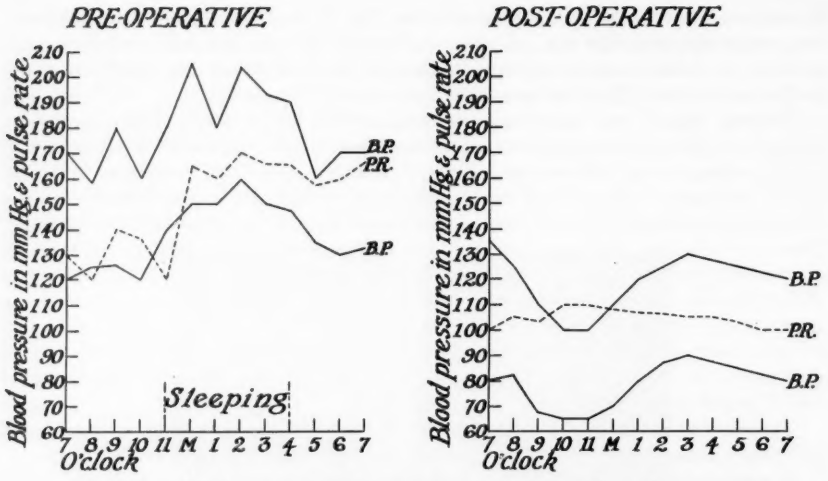
The effect of the sodium amytal sedation test, intravenous pentothal, epidural block and other medications on the blood pressure may be seen in Figures 2 and 3.

A specimen of venous blood was taken in a heparinized syringe while the blood pressure read 210/160 and the pulse rate was 160. Within three minutes, 1 cc. of this blood was tested on an isolated strip of rabbit's intestine suspended in Ringer's solution, with failure to demonstrate increased epinephrine content.

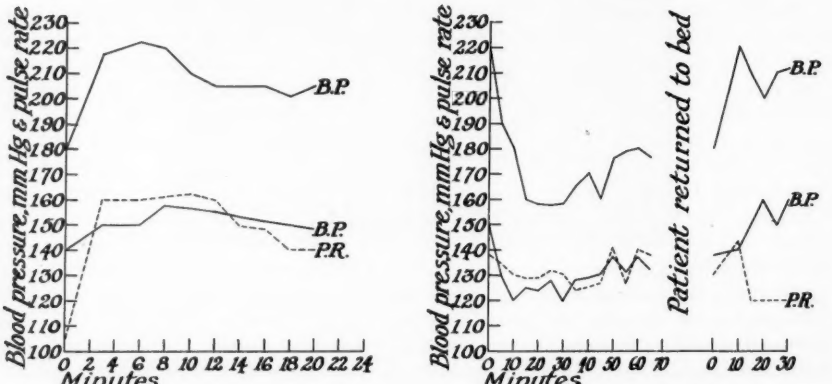
By the fifth hospital day the patient's vision had diminished so that she could discern only hand movements at 3 feet. The blood pressure, recumbent, was 185/135 and on assuming the standing position, 140/110.

On the ninth hospital day an *operation* was performed under anesthesia induced with cyclopropane and maintained with endotracheal ether. A posterior approach was used, with resection of a portion of the left twelfth rib. Through this incision a tumor mass could be felt lying above the kidney. On manipulation of the tumor the blood pressure rose to 190/180 and then 200/170, with the pulse staying at 160. As the tumor was removed, a precipitous fall in pressure occurred so that neither the blood pressure nor the peripheral pulse could be obtained. Through cannulated veins in the feet, blood was immediately transfused under pressure and 1 cc. of Neosynephrine was injected intravenously. This was followed by a return in pressure and a restoration of the pulse rate. Maintenance of an adequate pressure and pulse was accomplished by continuous infusion with blood, saline, and Neosynephrine. A clamp was left in place on the tumor pedicle and the patient was returned to her room in good condition. Percortin and adrenal cortical extract plus glucose and water were administered by venoclysis; the patient's condition remained satisfactory and the blood pressure and pulse were well sustained. On the day following operation she complained of vertigo, tinnitus, nausea and vomiting. Examination showed edema of the eyelids and hands. Adrenal preparations were discontinued and twenty-four hours later the vestibular symptoms and edema had subsided. In the ensuing two days the ileum became paralyzed and a superficial phlebitis of the left leg occurred at the infusion site. These complications responded readily to the usual measures.

Fundoscopic examinations were done daily and on the fifth postoperative day papilledema showed evidence of subsiding, less edema of the retina was noted and the arteries showed less spasm. Some organization of the macular star was now noted. Because her urine contained traces of sugar, a glucose tolerance test was performed, which showed

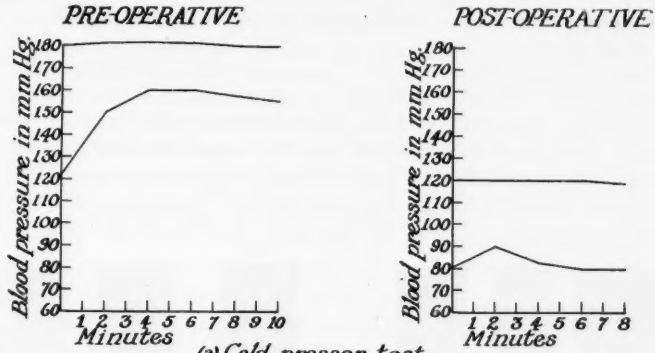


Sedation test one week pre-operative and 11 days post-operative, using 3 grains Sodium Amytal at 7, 8 and 9 P.M. respectively, and recording the Blood Pressure and Pulse Rate every hour. (through a misunderstanding the patient was awakened for blood pressure and pulse recordings during the post-operative test).



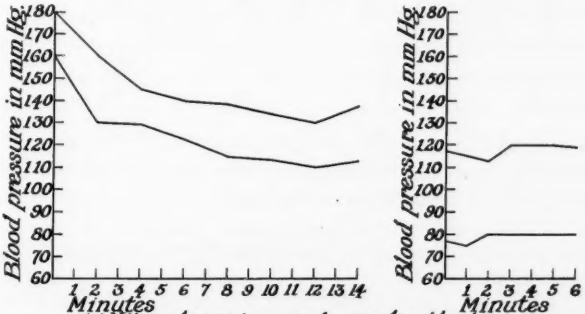
The effect of (a) 1.0 gram of Pentothal given intravenously as a 2½% solution, and (b) epidural block, on the blood pressure (B.P.) and pulse rate (P.R.) three days pre-operatively.

FIGURE 2



(a) Cold pressor test.

(b) Amyl nitrite pearl inhalation.



(c) Nitroglycerine, gr. $\frac{1}{200}$, under the tongue.

Response of blood pressure to (a) cold pressor test, (b) amyl nitrite pearl inhalation and (c) nitroglycerine gr. $\frac{1}{200}$ under the tongue on the day before operation and 11 days post-operatively.

FIGURE 3

a flat curve; fasting level, 78 mg.; one hour, 82 mg.; two hours, 73 mg.; and three hours, 88 mg., with all urine specimens free of sugar. On the eighteenth postoperative day the tumor mass on the right forearm was removed under local anesthesia and was reported to be a neurofibroma. She was discharged from the hospital on the nineteenth postoperative day. Her vision at this time was somewhat improved but was still not as good as on admission and she was able to read only the largest headlines of the newspaper and could not identify a person standing 4 feet from her. Two and one-half months later her blood pressure was 110/80 and her pulse was 76. There was no recurrence of her headache and her vision had improved markedly, so that she was able to read the

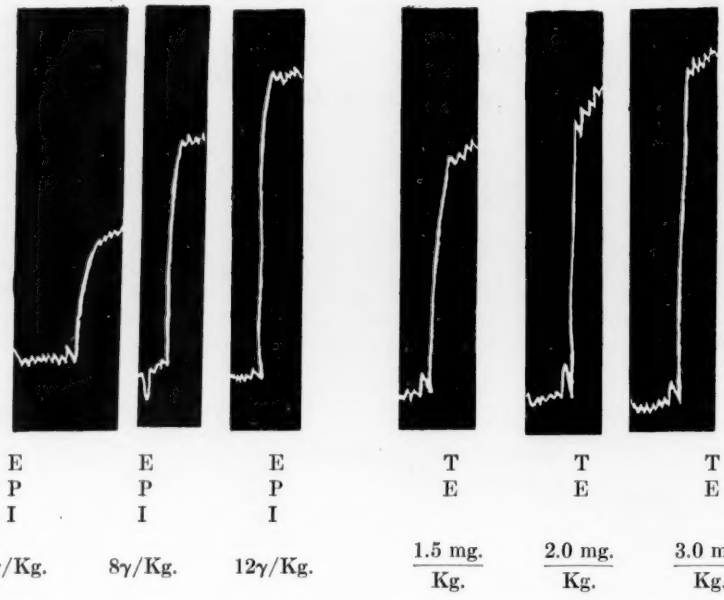


FIG. 4. On the left, the response of the atropinized cat's blood pressure to given doses of epinephrine (EPI) are shown. On the right is the response to graded amounts of tissue extract (TE). (Cat, 2.8 Kg. Anesthesia—Urethane.)

newspaper without difficulty. Six months later vision in the right eye was 20/30 and she had no further symptoms.

Examination of the excised tumor showed the following: The specimen consisted of a tumor measuring 5×5×3 cm., globoid in shape and weighing 54 Gm. It was encapsulated, soft, and brain-like. A cut section was pale grey and slightly mottled with hemorrhagic areas. The capsule contained yellowish adrenal cortical remnants. Microscopic sections showed characteristics of a chromaffin cell tumor. Extracts of the gland were tested chemically and physiologically and were found to give a response characteristic of epinephrine. The effect of tissue extract tested against a standard solution of epinephrine may be seen in Figure 4. From this, the epinephrine content of the tumor was estimated at 6 mg. per gram of tissue.

DISCUSSION

Although chromaffin cell tumors are known to cause paroxysmal hypertension with a normal pressure in the interim, it is only in the past few years that the importance of chronic hypertension in patients with this disease has been appreciated. As recently as October 1946, Washington *et al.* (9) stated "development of permanent hypertension is unusual in patients with this disease." Chronic hypertension has been noted however (1, 17, 18) and Green (4) in his analysis of 50 cases selected from the literature because of completeness of clinical or pathologic data, found that 37 patients were chronically hypertensive when first seen or became so in the course of varying periods of observation. Twelve of the 14 patients in the intermittently hypertensive group had their disease interrupted by surgical removal of the tumor, so that the likelihood of their becoming chronically hypertensive, while conjectural, seems likely. The importance of these observations cannot be overemphasized and in spite of the rarity of the disease, warrants the consideration of chromaffin cell tumor in the differential diagnosis of any case of hypertension, and especially in those suggesting malignant hypertension. Certainly the patient under discussion presented many of the criteria for a diagnosis of malignant hypertension, *e.g.*, the sustained high blood pressure, retinitis, urinary findings, loss of weight, and the sudden onset and rapid course of the disease in a young adult. However the very rapid pulse rate, the high basal metabolic rate, the abnormal response of the blood pressure and pulse to sedation tests, and the low left kidney as shown by retrograde pyelograms, served to point out the more probable diagnosis of chromaffin cell tumor.

In the case presented, the lowest pressures recorded preoperatively were 128 systolic and 103 diastolic and then only after inhalation of amyl nitrate. The sodium amytal sedation test on the other hand, resulted in a marked elevation of the blood pressure to 204/160 with the pulse varying between 120 and 170 (Fig. 2); and 1 gram of pentothal given intravenously resulted in a similar response, the blood pressure reaching 224/158 and the pulse jumping from 104 to 160. An epidural block caused some fall in pressure, but after moving the patient back to bed, a rapid rise occurred. The pressure and pulse noted during administration of intravenous pentothal might well be compared to the abnormal response to anesthesia or operation described under group 3; but in this case, fortunately, there was no progression to a more serious outcome. The cold pressor test provoked a sharp rise in diastolic blood pressure from 120 to 160 but had no effect on the systolic pressure; postoperatively no response was found in either systolic or diastolic pressures when the test was repeated (Fig. 3). This hyperactive response to cold pressor tests in patients with chromaffin cell tumor has

been noted previously (10, 11) and merely adds to the difficulties of interpretation of this test in any one hypertensive patient. The histamine test described by Roth and Kvale (16) was not attempted preoperatively because of the obvious danger in a patient whose vessels were already laboring under a very severe strain. Eleven days postoperatively, however, intravenous histamine elicited no blood pressure, pulse, or subjective response from the patient. Goldenberger's test (15) using Benzodioxane (933F) had not been described at this time.

There have been reports of severe retinal changes with these tumors (4) and Phillips (7) found in patients with chromaffin tumors that "in all patients with retinal changes there was persistent hypertension." In our case the retinal changes were grade IV and at the time of operation were progressing rapidly. In a six-month follow-up study these changes had nearly resolved and vision was 20/30 in the right eye. The vision in the left eye returned to 20/400 and the poor vision of this eye was attributed to a pre-existing amblyopia exanopsia (due to the squint and irregular astigmatism). At this time the only pathologic retinal changes were a faint outline of a macular star and a peculiar bronzed pigmentation at the periphery of the retina. This latter pigmentation appeared, as the edema and hemorrhage resolved. The mechanism of production of the retinal pigment in this case is not clear but brings to mind the close relationship between epinephrine and melanin, both being derived from the same mother substance or chromogen. In this same connection the frequent association of chromaffin cell tumors and Von Recklinghausen's disease has been previously noted (2, 8). This patient had many "cafe au lait" spots on the body as well as a neurofibroma of the right forearm and a familial history of cutaneous pigmentation. It would therefore appear reasonable to place her in the group of patients having a variant of Von Recklinghausen's disease. (An unsuccessful attempt was made to ascertain the pathologic nature of the tumors removed from her left upper eyelid ten years previously. The possibility that these were neurofibromas seems likely.)

The preoperative and postoperative care of these patients, when subjected to surgery, is outlined by Biskind, *et al.* (19). Although the use of adrenal cortical extract, desoxycorticosterone acetate and a high salt diet before and after operation is recommended, several cases have been reported in which edema or other signs of fluid retention have occurred (4, 7) and it is necessary that care be taken to avoid this complication. The edema and vestibular symptoms which developed in our patient were undoubtedly caused by the hormonal imbalance induced by treatment; they disappeared promptly on withdrawal of the adrenal substances. When the diagnosis of chromaffin tumor is entertained and an operation is to

be performed, a cutdown on at least one vein (and preferably two) should be performed so that fluids and medications, especially epinephrine or Neosynephrine, may be administered as needed. This is exceedingly important, for when the need arises, it is immediate and any delay at this point may result in the loss of the patient. Green (4) found it necessary to administer life-saving fluids and drugs intrasternally when his patient went into shock, for intravenous medication including cutdowns were impossible. In our own case we were prepared for the sudden shock which so often follows manipulation and removal of these tumors, and had placed needles in cutdowns on the veins in both feet. At the moment of shock however, when the urgent need had arisen, one of the needles became completely unserviceable. If the other needle had not been in place, an irremediable situation might well have developed.

SUMMARY

1. A case of chromaffin cell tumor simulating malignant hypertension in a patient with a congenital urinary tract abnormality and Von Recklinghausen's disease, is described and discussed.

2. The importance of considering a chromaffin cell tumor in the etiology of chronic hypertension is stressed.

3. Relief of the symptoms by surgical removal of the tumor is described. Mention is made of certain precautions to be taken in the care of the patient at the time of operation.

Acknowledgment

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Endocrine Review

METHYLANDROSTENEDIOL

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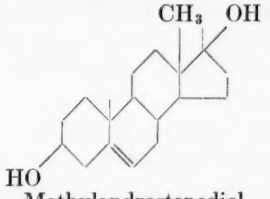
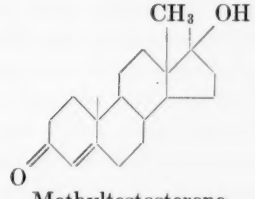
METHYLANDROSTENEDIOL is a steroid that was first synthesized in Ruzicka's laboratory (1) in 1935. Since it exhibits some unusual properties, renewed interest has been aroused in recent months, and in view of this we have undertaken to compile the present survey of the data available to us.

Although its behavior is rather different, methylandrostenediol is most closely related to methyltestosterone, the structural difference being an alcohol rather than a ketone at carbon 3, associated with the characteristic shift of the double-bond from 4:5 to 5:6. The respective structural formulae may be compared:



Methylandrostenediol



Methyltestosterone

One readily supposes that the compound, in metabolism, might be modified to become methyltestosterone and in this way some, but not all, of its behavior can be explained. Quite different modifications have also to be considered.

The intensity of its several actions is found to vary considerably in animals, depending upon whether it is administered orally or parenterally; and the variation does not have the simple quantitative character, seen with many steroids, that oral administration is merely less efficacious. Contrariwise, methylandrostenediol on oral administration exhibits a moderate androgenic activity and a capacity to inhibit certain experimental tumors, which properties disappear or are impaired on subcutaneous injection. When given parenterally to the experimental animal there is a well marked protein-anabolic action which is only poorly manifest when

the substance is given by mouth. Some other properties might also suggest that two or more different steroids are involved, depending upon the metabolic history of the single substance administered. Whereas the literature has dwelt at length upon conjectured interconversions among steroids, and indeed these have afforded a rather facile interpretation of experimental findings, it does appear that in the case of methylandrostenediol an explanation in these terms is well founded.

A protein-anabolic steroid devoid of specific sexual activity has been sought for some years. Although laboratory data in animals suggest that methylandrostenediol may have this property, it needs to be further studied in human subjects before applying the animal data to the human. It is well known, of course, that testosterone is a powerful protein-anabolic agent, but its widespread use has been handicapped by the potent virilizing action. One satisfactory solution has been the use of testosterone and estradiol simultaneously for protein-anabolic therapy, although the proportions need to be carefully adjusted for weight, sex and individual. The prospect that methylandrostenediol may serve as a satisfactory anabolic agent is sufficiently attractive to warrant extended studies.

CHEMISTRY

The compound here described is that isomer which is directly correlated with methyltestosterone, namely, 17α-methyl-Δ^5-androstene-3β, 17β-diol. Despite confusion due to the older assignment of *trans* or α position to the 17-hydroxyl group in the androgens, it seems clear that all references to methylandrostenediol, not otherwise specified, signify this isomer.[1] The empiric formula is $C_{20}H_{31}O_2$, the molecular weight is 303.25. This compound occurs as a white crystalline substance, insoluble in water, petroleum ether and benzene, but soluble in chloroform, pyridine and boiling methanol. Its melting point is 199–206° C., or 204° C. (1), or 203–204° C. (2), or 205.5–206.5° C. (3). The optical rotation (3) is $[\alpha]_D = -78$ to -84 (1 per cent in dioxane), or -73. Some of the esters which serve for identification are the 3-acetate, m.p. 174–180° C. or 179–180° C. (3) $[\alpha]_D = 72$ (3); the 17-acetate, m.p. 164° C. (4); the diacetate, m.p. 145–146° C. (3), $[\alpha]_D = 59$ (3); the 17-propionate, m.p. 138°; dipropionate, m.p. 83° C. (4).

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[1] This compound is identical with Selye's (5) No. 255, *then* called "methyl trans-androstene-diol" or 17β-methyl-Δ^5-androstene-3β 17α-diol. The compound *now* properly given that name is the 17-iso compound (3), m.p. 203–204, $[\alpha]_D = -81$, 3-acetate, $[\alpha]_D = 77$ and m.p. 160–161, forming no diacetate (attributed (3) to steric hindrance by 17-methyl in *cis* position). Miescher (3) distinguished the isomers as the *normal* and the 17-iso compounds. The present statement refers to the normal compound.

TOXICITY

Acute toxicity: This has not been ascertained quantitatively, since it was not practicable to give enough of the compound (Methostan, Schering) to produce death in mice or rats. No death occurred following oral or subcutaneous administration of 9 Gm./Kg. in rats, oral administration of 9 Gm./Kg. in rats or mice, or intraperitoneal injection of 6 Gm./Kg. in mice. The LD_{50} in these species therefore exceeds these dosages by an undetermined amount (6).

Chronic toxicity: Aqueous suspensions of the compound were fed to female rats in dosages of 225 to 900 mg./Kg. daily for six weeks without significant change in body weight in the treated *vs.* the control animals. Diestrus was present in the treated animals and may have been attributable to oral androgenic activity of the compound.

Subcutaneous injections into female rats of 75 to 300 mg./Kg. daily, in aqueous suspension, caused some depression in the rate of growth during six weeks, but no animals died, and it is believed that the distress attending these large injections, rather than any action of the compound, was responsible for the difference (6). Subcutaneous injections of up to 75 mg./Kg. daily in male rats likewise led to retardation of growth.

Local toxicity: The compound was compared with testosterone; 0.2-cc. portions of aqueous suspensions of 5.6 mg./cc. were injected subcutaneously daily for six days into female rats. No significant irritation was visible grossly or on microscopic study with either compound. Subcutaneous injection of 0.2 cc. of 10 per cent aqueous suspension into mice (weighing 18–22 Gm.) did not produce necrotic changes within two weeks (6).

Toxicity in man: Excepting 1 instance of gastro-intestinal irritation in a girl with Addison's disease (7), a result which has never been duplicated in any of some 60 patients, the compound has given no evidence of toxicity in man when administered orally, parenterally, sublingually or by implantation of pellets, in the dosages to be cited. Reference has been made (8) to a possibility of hypercalcemia following treatment with the compound, in patients with mammary cancer metastatic to bone, an effect that has been noted with other steroids in a small number of cases; it is not possible to conclude whether or not it is more likely to occur with this compound than with others. In the usual range of dosage, toxicity of the compound is not a clinical consideration.

HORMONAL ACTION

From the time it was first prepared (1), methylandrostenediol was regarded as an androgen, the capon unit corresponding to about 0.65 mg., and the compound having, therefore, about one fiftieth the activity of tes-

tosterone (1). Comparisons of the effect on the seminal vesicles and prostate of the rat (1, 5, 9, 10, 11, 12) likewise showed some definite androgenic activity, much less than that of testosterone, but greater than that of androsterone (9). Slight estrogenic (10) and progestational (13, 14) actions were also found. There was some antagonism to the testis-damaging effect of estradiol (5), and there was relatively prominent "anesthetic" action in hepatectomized rats and in fish, the compound being about one twenty-fifth as active as pregnanedione in the rat (5). Other studies have elicited a spermatogenic action (2), and also a protein-anabolic action—which is characteristic of potent androgens but which has been present, in most other compounds, somewhat in proportion to the androgenic activity. The compound did not have glycogenic (adrenocortical) activity when tested for antagonism to insulin in the adrenalectomized rat (15).

Androgenic action: On oral and on subcutaneous administration, in immature Sprague-Dawley rats of 53–60 Gm., taking the weight of the seminal vesicles as a criterion, the compound was found to have about one third the activity of testosterone, subcutaneously, and about the same activity as methyltestosterone, orally (6):

Steroid	Doses required to increase seminal vesicle wt. to 40 mg.
	mg. per rat
Subcutaneous administration	
Methylandrostenediol	0.90
Methyltestosterone	0.34
Oral administration	
Methylandrostenediol	19.1
Methyltestosterone	16.6

Oral and subcutaneous activities were compared likewise, using the comb response in day-old cockerels. The oral material was introduced into the diet; the parenteral material was given as an aqueous suspension. On subcutaneous administration, methyltestosterone in a dose of 2.5 mg. every three days gave a positive response after seven days, whereas 5 mg. of methylandrostenediol was ineffective after twenty days (4 doses, on days 1, 4, 8, 18). Feeding the two compounds at a computed daily dosage of 4 and 10 mg., respectively, produced comb responses after eleven days (positive results in all chicks). No evidence of anabolic activity in this species appeared in the experimental data, for either compound by either route, at this dosage (6).

The data suggest that oral methylandrostenediol is 0.4 to 1.0 times as active as oral methyltestosterone; but that parenteral methylandrostenediol is rather weakly androgenic—from 0.02 (capon, (1)) to 0.3 (rat, (6)) times as active as testosterone. The species differences are of practical interest, as it has been shown clinically (*see* subsequent section) that the oral androgenic activity for man is far lower than 0.4 times that of methyltestosterone. When methylandrostenediol was given by intramuscular *injection* into malnourished, maldeveloped male infants, in doses of 50 mg., 100 mg. and finally 200 mg. daily, there was a total absence of androgenic effect, whereas the same infants responded immediately to 25 mg. of testosterone propionate daily by injection (16).

Spermatogenic action: Comparatively few steroids enable hypophysectomized animals to maintain spermatogenesis; pregnenolone is outstanding among these, at least experimentally, an equivalent clinical action being thus far unavailable. When immature rats were injected twice daily for ten days, beginning the day after hypophysectomy, methylandrostenediol was effective in maintaining spermatogenesis (2):

Treatment	Testis, wt., mg.	Seminal vesicles, wt., mg.	Prostate, wt., mg.	No. of rats having spermatozoa
Methylandrostenediol, 2 mg./day	378	152	157	4 out of 5
Testosterone, 2 mg./day	310	143	187	0 out of 7
Cholesterol	105	3.7	10.2	0 out of 11
Intact, untreated controls	878	10.9	57	10 out of 10

Other data (17) make it clear that a definite spermatogenic action is regularly elicited with testosterone propionate in doses one-twentieth as great as this; and all other results indicate a much lower degree of androgenic activity for methylandrostenediol than appeared in this study (2). Not only is interpretation therefore difficult, but evaluation of the effect of this compound in clinical oligospermia must be made independently of results secured in lower species.

Estrogen antagonism: A 2-microgram dose of estrone subcutaneously is found uniformly effective in cornifying the vaginal smear of castrated, mature Sprague-Dawley rats in this laboratory. When methylandrostenediol and testosterone were compared for antagonism to this effect, on subcutaneous injection, the former compound was shown to be much less active (1:5) than testosterone. When compared with methyltestosterone, on oral administration, it was half as active (6).

An older test of estrogen antagonism (5) utilizes the weight of the testis

in rats which have received large doses of estradiol. When methylandrostenediol was tested in this way, more than 50 per cent increase in testis weight was found with a dosage of 1 mg. by injection. Of all steroids so tested, only androstenediol was more active, a comparable weight change being secured with 0.5 mg. Oral administration was not employed in these studies.

Progestational activity: When immature female rabbits are primed with 9 micrograms of estradiol benzoate (total dosage intramuscularly during five days) and then given methylandrostenediol by mouth, progestational changes appear in the uterus in relation to the dosage used, ranging from greater than 3.5 (McPhail index) with 32 mg. to less than 1.5 with 4 mg. of the compound. The same procedure carried out simultaneously using ethinyltestosterone (anhydrohydroxyprogesterone) as the progestational agent shows that methylandrostenediol and ethinyltestosterone have closely similar activity when given by mouth to rabbits, possibly higher grades of response being reached with smaller amounts (*e.g.*, 16 mg.) of ethinyltestosterone. By injection, methylandrostenediol is only feebly active (13, 14) compared with progesterone (14), about in the proportion 1:30.

Estrogenic activity: This has been demonstrated in limited degree (10).

Effects on tissue enzymes: Alkaline phosphatase of liver and spleen, in the rat, is increased by the subcutaneous (but not oral) administration of methylandrostenediol, whether the animals be intact, adrenalectomized or hypophysectomized; but the last-named group is more sensitive to this action (6).

Anti-neoplastic action: Sarcoma 180 and mammary gland adenocarcinoma C3HBA in mice, initiated by 1–2 mm. subcutaneous implants in the axilla, were retarded by feeding 0.03 per cent or 0.06 per cent of methylandrostenediol, in the diet in either males or females. In these animals the compound was mixed with the standard maintenance ration (Wayne-Fox food) in solution in corn oil or in aqueous suspension, beginning the day after implantation; but some (lower) effectiveness remained when administration of the compound was delayed for five days after implantation. Since the mice ate about 3 Gm. of food daily, the effective dose of methylandrostenediol was about 2 mg., or 100 mg./Kg. daily, by mouth. Comparable results followed 100 mg./Kg. of methyltestosterone given by mouth. Subcutaneous injection of methylandrostenediol, 50 and 100 mg./Kg. daily, *did not* result in inhibition of tumor growth (18).

Protein-anabolic action: Nitrogen balance was improved in castrate dogs by as little as 6 mg. (av.) daily by intramuscular injection; but oral dosage of 200 mg. daily did not lead to the retention of nitrogen by the dog. Similarly, a protein-anabolic action in respect to renal weight, of small

but significant degree, resulted in mice implanted with pellets of the compound. At the dosage used, calculated at 1 mg. per day for thirty days, there was no observable androgenic action; and 7.5 mg./Kg. daily by mouth in mice produced no increase in kidney weight but definite enlargement of the seminal vesicles. In these two species, then, the compound has anabolic action when given parenterally but not when given by mouth.

Oral vs. parenteral activity: The following table summarizes the oral and parenteral effects and shows the contrasts between them in several species of animals (6). Conclusions should not be extended to man, of course, until supported by specific data. Since methyl androstenediol is very poorly soluble in most organic solvents suitable for injection, consideration must be given to the possibility that aqueous suspensions of the steroid given parenterally may be very poorly absorbed in certain species depending upon the site and manner of injection, thus accounting for the striking difference in activity between the oral and parenteral materials.

THE ROLE OF THE ROUTE OF ADMINISTRATION ON THE ACTIVITY OF METHYLANDROSTENEDIOL

Test	Oral	Parenteral
Anti-tumor, in mice:		
Sarcoma 180	Active at 100 mg./Kg.	Inactive at 100 mg./Kg.
Mamm. carcinoma	Active at 100 mg./Kg.	Inactive at 100 mg./Kg.
Anabolic:		
Castr. dog	Inactive (200 mg./day for 14 days)	Active (12.5 mg. i.m. on alternate days, oil susp.)
Castr. male mice	Feebly active at 1.5 mg./Kg.	Active (pellet implants, absorption: 1 mg. in 30 days)
Androgenic:		
Male rat	$\frac{1}{2}$ to $\frac{7}{8}$ of methyltestosterone activity	$\frac{1}{5}$ of testosterone activity
Castr. male mice	Active at 1.5 mg./Kg. per day for 30 days	Inactive (pellet implants, absorption: 1 mg. in 30 days)
Chick comb	Active at 100 mg./Kg.	Inactive at 100 mg./Kg.
Estrogen inhibition:		
Castr. female rat	$\frac{1}{2}$ of methyltestosterone activity	$\frac{1}{6}$ of testosterone activity
Progestational:		
Rabbit	Equal to anhydrohydroxyprogesterone	Very slight (1/10 to $\frac{1}{3}$ of progesterone activity)
Acute toxicity: LD_{50}	>9.0 Gm./Kg.	>9.0 Gm./Kg.

CLINICAL

Cancer: Homburger, Forbes and Desjardins, recognizing the combination of anabolic, kidney-protective and androgenic properties displayed by testosterone, and having devised a rather direct, comprehensive assay for this kind of physiologic spectrum (19), noted with interest that methylandrostenediol conformed closely and at least warranted appraisal as a palliative steroid for the treatment of malignant disease. Homburger, Kasdon and Fishman (20) administered up to 100 mg. per day parenterally to 7 women with mammary cancer metastatic to bone, for periods of one to seven months. Regression of some metastases and demonstrable recalcification of bone were observed, in addition to euphoria; and 3 or more of the patients could be regarded as having general benefit. No masculinizing action was observed at this dosage.

The more definitive report of Kasdon, Fishman and Homburger (21) now embodies 24 cases of female breast cancer treated by intramuscular injection in dosages varying from 25 mg. per day (in oil) to 100 mg. per day (aqueous suspension) and by implantation of pellets subcutaneously, with objective as well as subjective improvement in 6 and subjective benefit in 12 of the patients. The largest total dose was 19.4 Gm. given over a period of twelve months. Although some growth of facial hair was seen in a single instance, the other women did not exhibit masculinizing effects, and 1 patient who had become amenorrheic with testosterone resumed cyclic menstruation while receiving methylandrostenediol. Evaluation of the compound in terms of survival or prolongation of life in this disease has, appropriately, not yet been attempted. The impression seems warranted, however, that palliation comparable with that given by testosterone can be secured without the distraction of intense androgenic activity.

In the light of the animal experiments, it is of great interest to inquire into the effect of *oral* administration of methylandrostenediol in patients with malignant disease. The Memorial Hospital group (22) treated 5 women with mammary cancer, using 100 mg. daily by mouth. Treatment was discontinued after a week in 1 patient, because of "increased respiratory difficulty." In 2 patients there was symptomatic improvement (alone) during two months of treatment. The remaining 2 patients, having osseous metastases, gave evidence of a definite intercession of methylandrostenediol in bone metabolism. One was a woman aged 41 years, castrated by roentgen rays two years earlier, who had considerable symptomatic benefit associated with an increase of serum alkaline phosphatase from 12 to 19 Bodansky units in six weeks. The other patient had received a testosterone compound up to one month previously, so that regression in the bone disease during the first two weeks of methylandrostenediol therapy was still

attributable to the earlier treatment. However, after nine weeks, and a total dosage of 8,200 mg. of methylandrostenediol, the syndrome of hypercalcemia developed, with a serum calcium of 16.3 mg. per cent, and increased bone pain. Slow improvement was noted on discontinuing methylandrostenediol.

Protein anabolic action: Methylandrostenediol may be estimated to have about 0.2 times the protein anabolic activity of testosterone; but since its relative androgenic potency is considerably lower, it presents a potential advantage for the treatment of such conditions as malnutrition and cachexia, with lesser or no masculinizing effect. This estimate is based on the following clinical data:

An Addisonian girl of 8 years who was maintained by 3 mg. of desoxycorticosterone acetate and 9 Gm. of salt (NaCl) daily, retained nitrogen in excess of 0.1 Gm./Kg. per day during treatment with 90 mg. of methyltestosterone by mouth or 25 to 50 mg. of testosterone propionate by injection, daily. With 50 mg. methylandrostenediol by mouth, she retained 0.05 Gm./Kg. of nitrogen daily, but did not gain weight (7). This girl was remarkable in that her mouth became sore and she passed mucous stools during the time the compound was administered and until shortly after it was stopped. Apparently either an individual reaction or the basic disease was responsible for these side-actions, which have not been encountered in other cases.

In 3 male dwarfs (2 panhypopituitary dwarfs aged 17 and 15 years, and a boy of 12 with Fröhlich's syndrome, retarded growth and obesity) (23), nitrogen was retained to the same extent as in the patient just described, in 1 instance, but not markedly in the others:

Diagnosis	Panhypopituitarism				Fröhlich
Age (in years)	17		15		12
Weight—Kg.	43		32		40
Diet: protein Gm./Kg.	1.4		1.5		1.0
calories per Kg.	48		49		25
Dosage: oral, mg. per day	30	30		40	10–20
Dosage: intramusc., mg. per day		7.5	10		
Days treated	21	15	24	24	16
Nitrogen retained, mg./Kg.					
Control period	14	14	34	34	18
Treatment period	45	51	31	25	25

"No androgenic effects or toxic symptoms were noted. There were definite weight gains."

In an early report, Thorn (24) also demonstrated that methylandrostenediol has a very definite effect on protein anabolism. In average daily doses of 22.3 mg. given both by oral administration and intramuscular injection, 4 patients who were given the steroid for twenty days showed average nitrogen retention per kilo of body weight per day, as follows:

Days 1 to 5—20 mg.
Days 5 to 10—25 mg.
Days 10 to 15—25.5 mg.
Days 15 to 20—23.5 mg.

This author did not observe any signs of masculinization in these patients with the dosage used and for the period of time studied. He stated, however, that since methylandrostenediol was a weak androgen by bioassay, it was quite probable that masculinization would occur if the dosage were sufficiently increased.

A normal woman aged 42 years, receiving 25 mg. daily (by intramuscular injection?) for six days, showed about half the protein anabolic effect that was induced by a like dosage of testosterone propionate (20).

In addition to studying the effect of methylandrostenediol on breast cancer, Kasdon *et al.* studied its protein-anabolic effects in cancerous as well as noncancerous patients. These workers observed no masculinization in their patients and concluded that methylandrostenediol is a weakly-virilizing, nontoxic agent with protein-anabolic effects.

In view of the anabolic ineffectiveness of 17-methyl androgens as exemplified by methyltestosterone, in the presence of thyroid overactivity, it should be supposed for the present that the compound would not be a desirable agent in hyperthyroidism.

Creatinuria: In general, the 17-methyl androgens give rise to creatinuria. In 5 patients receiving methylandrostenediol, 30 to 50 mg. orally or 10 mg. intramuscularly daily for sixteen to forty-five days, the increase in creatinuria due to treatment was very marked (25). Nitrogen retention was shown, in the same study, to be less than that with testosterone or methyltestosterone, graded +, but greater than that with several other androgens, graded 0; the compound was assigned the grade ±, and the anabolic effect at this dosage was declared "of doubtful significance."

Arthritis: The compound has been administered orally to patients with rheumatoid arthritis. Six women received 200 mg. daily for two weeks and then 400 mg. daily for ten days, without influence upon the disease, and without producing androgenic effect or disturbance of the menstrual cycle. One man with spondylarthritis likewise did not respond to 400 mg. daily given for two weeks, although his general condition was improved and he gained weight (26). These results may be taken as evidence that compar-

atively large oral doses of the compound are not effectively androgenic in man and are nontoxic.

SUMMARY

The evidence presented tends to show that methylandrostenediol is a feeble androgen when compared with the standard testosterone propionate. It also shows that this steroid has a definite protein-anabolic effect if given in sufficient dosage. Preliminary reports indicate also that it may be useful for the palliative treatment of certain types of female breast cancer. Further carefully controlled studies are necessary to show the most efficacious method of administration and the potential clinical usefulness in many conditions in which its effect has not yet been investigated.

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Letter to the Editor

THE PRECIPITATION OF HEMORRHAGE BY STRESS IN CERTAIN DISEASES WITH BLEEDING DIATHESES

To the Editor

Adrenal cortical augmentation of hemostasis is indicated by the following clinical observations made upon three hemorrhagic diseases, of divergent types:

Hemophilic arthropathies and hematuria had been the principal manifestations of disease in a 14-year-old boy, who had been known to have been a bleeder since birth. Crippled from knee contractures, anemic, underdeveloped sexually and subject to at least weekly joint "flareups," he underwent blood studies in spite of his deep-seated fear of the punctures required. Hematuria began the following day, persisted for several weeks, and occurred again when he was required to wear plaster casts, which were applied to correct the knee contractures. Cortisone therapy was started at this time with prompt, coincident remission of the hematuria. Since then he has been admirably maintained upon this regimen, with no further hematuria; control of the joint involvement was obtained within three months and was adequate during the subsequent four months. General health improved proportionately, although the Lee-White blood coagulation time was not appreciably reduced (one hour, plus).

Stress has appeared to be an exciting factor in the melena of a woman with disseminated telangiectasis. Her emotional upsets were dramatic enough to indicate such a relationship in the past, further substantiated by the Thorn test, by the flicker photometer test and, apparently, by the cortisone response. Under cortisone therapy the number of transfusions required have been reduced from two or three a month to three in the past seven months.

Using the tests just mentioned, a man of 64 years, who had had two massive hemorrhages from a duodenal ulcer, was rather closely followed for evidence of stress. Three days before his last (severe) hemorrhage, the circulating eosinophils dropped 50 per cent from their previous average, and the flicker fusion time became plus 240 after nitroglycerin. Although the temporary use of ACTH to bring about amelioration of the stress has much to recommend it, this treatment was not given because of the adverse opinions from authoritative sources. An unusually rapid recovery however, was coincident with the use of moderate amounts of cortisone, continued until his red blood cell count had reached four million (from a low of two million).

Thrombo-embolic complications, attributed to the use of cortisone, have been reported from various sources; the recorded experiences with these hemorrhagic disorders would seem to lend weight to theories as to this

function of cortisone. Selye has described the "fluidity" of the blood during the "alarm" (stress) phase of his adaptation syndrome, which stage is counteracted or adapted by adrenal cortical activity.

It is hoped that statistically valid studies may be undertaken to clarify the endocrine aspects of hemostasis.

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The Association for the Study of Internal Secretions

THE 1951 ANNUAL MEETING

GENERAL INFORMATION

Headquarters: Chalfonte-Haddon Hall, Atlantic City, New Jersey

Registration: Everyone attending the meetings is required to register. By action of the Council, non-members will be charged a registration fee of $2.00 for any part of the scientific sessions. Members should present their current membership cards when registering. Admission will be by badge only.

The Scientific Sessions: Due to increasing the meeting to three days this year, all scientific sessions will not be held in the same room. Please refer to the daily program as to the location of each session. Programs will begin promptly on schedule. Presentation of papers at all sessions will be LIMITED TO TEN MINUTES and authors are requested to adhere strictly to this schedule. Manuscripts of all papers should be submitted to the presiding officer or to the Secretary-Treasurer at the end of the presentation.

Annual Dinner: The Annual Dinner of the Association will be held on Friday evening, June 8, at 7:30 P.M. in the Rutland Room of Chalfonte-Haddon Hall preceded by cocktails at 6:30 P.M. in the Benjamin West Room. Secure tickets at time of registration.

Local Arrangements: Dr. Matthew Molitch, 705 Pacific Avenue, Atlantic City, New Jersey, is in charge of the local arrangements for the meetings.

Secretary-Treasurer: Dr. Henry H. Turner, 1200 North Walker Street, Oklahoma City 3, Oklahoma.

(For the program *see* the May issue of the Journal)

Fourth Annual Postgraduate Assembly in Endocrinology Including Diabetes

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THE AMERICAN DIABETES ASSOCIATION

SEATTLE, WASHINGTON OLYMPIC HOTEL JULY 2–7, 1951

The faculty will consist of prominent researchers and clinicians in the field of endocrinology and metabolic disorders.

The course will be a practical one of interest and value to the specialist and those in general practice. The program will consist of lectures, clinics, and demonstrations. Ample time will be given to questions and answers at the end of each session, and registrants are encouraged to contact members of the faculty for individual discussions.

This program has been accepted for credit by The American Academy of General Practice.

Due to certain regulations, the 1951 course will not be available to veterans under Public Law 346.

There will be sight seeing, teas, and other entertainment for the ladies. Bring your family. Many interesting and enjoyable short tours are available out of Seattle, including trips to Cascade Mountains, Mt. Rainier, Chinook Pass, and Olympic National Park. Western Canada's scenic wonderland including Victoria, Vancouver, Jasper, Banff, and Lake Louise are only a short distance away.

The Olympic, one of Seattle's most delightful hotels, offers special convention rates to members of this assembly. This is an unusual opportunity for you and your family to enjoy a pleasant vacation in the beautiful Pacific Northwest and for you to participate in a highly instructive program of the latest advances in endocrinology and metabolism.

A fee of $75 will be charged for the entire course and the attendance will be limited to 100. REGISTRATION WILL BE IN THE ORDER OF CHECKS RECEIVED AND WILL CLOSE ON JUNE 4, 1951. SHOULD THERE BE AN INSUFFICIENT NUMBER OF APPLICANTS TO FILL THE COURSE, THE REGISTRATION FEE WILL BE REFUNDED IMMEDIATELY IN ITS FULL AMOUNT.

Please forward application on your letterhead, together with check payable to The Association for the Study of Internal Secretions, to Henry H. Turner, M.D., Secretary-Treasurer, 1200 North Walker Street, Oklahoma City 3, Oklahoma, *before June 4, 1951*. Further information and program will be furnished upon request.

Hotel reservations should be made directly with the Olympic Hotel, Seattle, Washington, and the hotel advised that you are attending this Postgraduate Assembly.

FACULTY

Dr. Alexander Albert, Consultant and Head, Endocrine Laboratory, and Assistant Professor of Physiology, Mayo Clinic, Rochester, Minn.

Dr. Willard Allen, Professor of Obstetrics and Gynecology, Washington University School of Medicine, St. Louis, Mo.

Dr. Russell D. deAlvarez, Professor and Executive Officer, Department of Obstetrics and Gynecology, University of Washington School of Medicine, Seattle, Wash.

Dr. Elmer C. Bartels, Staff Member, Department of Internal Medicine, Lahey Clinic, New England Baptist Hospital and New England Deaconess Hospital, Boston, Mass.

Dr. Peter Forsham, Instructor in Medicine, Harvard Medical School and Junior Associate in Medicine, Peter Bent Brigham Hospital, Boston, Mass.

Dr. Carl Heller, Associate Professor of Physiology, Medicine and Endocrinology, University of Oregon School of Medicine, Portland, Ore.

Dr. Blair Holcomb, Clinical Professor of Medicine, University of Oregon School of Medicine, Portland, Ore.

Dr. Brien T. King, Senior Consultant in Surgery, University of Washington School of Medicine, Seattle, Wash.

Dr. Hans Lisser, Clinical Professor of Medicine, University of California, San Francisco, Calif.

Dr. Francis Lukens, Associate Professor of Medicine, University of Pennsylvania School of Medicine and Director, George S. Cox Institute, University of Pennsylvania, Philadelphia, Penn.

Dr. E. Perry McCullagh, Head, Section of Endocrinology and Metabolism, Cleveland Clinic, Cleveland, Ohio.

Dr. D. F. McDonald, Assistant Professor of Surgery, Urological Unit, University of Washington School of Medicine, Seattle, Wash.

Dr. Lester Palmer, Clinical Professor of Medicine, University of Washington School of Medicine and Department of Medicine, Mason Clinic, Seattle, Wash.

Dr. Harold D. Patton, Associate Professor, Department of Physiology and Biophysics, University of Washington, Seattle, Wash.

Dr. Edward C. Reifenstein, Jr., Director, Oklahoma Medical Research Institute, Oklahoma City, Okla.

Dr. Henry Ricketts, Professor of Medicine, University of Chicago, Chicago, Ill.

Dr. Arthur L. Rogers, Clinical Instructor of Medicine, University of Oregon School of Medicine, Portland, Ore.

Dr. Howard Root, Associate Professor of Medicine, Harvard University Medical School, and Physician-In-Chief, New England Deaconess Hospital, Boston, Mass.

Dr. Homer Rush, Clinical Professor of Medicine and Cardiology, University of Oregon School of Medicine, Portland, Ore.

Dr. E. Kost Shelton, Director of the Endocrine Clinic, Los Angeles General Hospital, Los Angeles, Calif.

Dr. James Sherrill, Medical Director, Scripps Metabolic Clinic, La Jolla, Calif.

Dr. Kenneth Swan, Professor of Ophthalmology and Head of Department of Ophthalmology, University of Oregon School of Medicine, Portland, Ore.

Dr. Henry H. Turner, Clinical Professor of Medicine, University of Oklahoma School of Medicine, Oklahoma City, Okla.

Dr. Lawson Wilkins, Associate Professor of Pediatrics, Johns Hopkins University, Baltimore, Md.

Dr. Robert H. Williams, Professor and Executive Officer, Department of Medicine, University of Washington School of Medicine, Seattle, Wash.

Dr. Ben Vidgoff, Clinical Associate, Pharmacology, University of Oregon School of Medicine, Portland, Ore.

PROGRAM

Monday, July 2, 1951

8:00– 9:00 REGISTRATION

GENERAL, THYROID, ADRENALS

9:00– 9:20 General Principles of Endocrinology—Dr. Forsham
9:20– 9:40 Pituitary Physiology and Chemistry—Dr. Williams
9:40–10:00 Thyroid Physiology—Dr. Albert
10:00–10:20 Diagnostic Tests of Thyroid Disease—Dr. Bartels
10:20–10:30 Intermission—Refreshments
10:30–11:00 Treatment of Thyrotoxicosis—Dr. Bartels
11:00–11:10 Treatment of Myxedema—Dr. McCullagh
11:10–11:20 Diagnosis and Treatment of Hypometabolism—Dr. McCullagh
11:20–11:50 Diagnosis and Treatment of Non-toxic Goiter and Cancer; Tumors of Neck—Dr. Bartels
11:50–12:00 Diagnosis and Treatment of Thyroiditis—Dr. King
12:00–12:45 Case Presentations
12:45– 2:00 Luncheon
2:00– 2:30 Questions and Answers—Dr. Williams, Moderator
2:30– 3:00 Adrenal Physiology—Dr. Forsham
3:00– 3:30 Adaptation Syndrome—Dr. Reifenstein
3:30– 3:45 Intermission—Refreshments
3:45– 4:15 Diagnostic Tests of Adrenal Function—Dr. Albert
4:15– 4:35 Diagnosis and Treatment of Cushing's Disease—Dr. Bartels
4:35– 5:05 Diagnosis and Treatment of Acute and Chronic Adrenal Insufficiency—Dr. Forsham
6:30 Get Acquainted Party—Cocktails and Dinner

Tuesday, July 3, 1951

ADRENALS, TESTES, OVARIES

9:00– 9:30 ACTH and Cortisone—Dr. Albert
9:30–10:00 ACTH and Cortisone—Dr. Forsham

10:00–10:50 Diagnosis and Treatment of Adreno-genital Syndrome, Hirsutism and Intersexuality—Dr. Wilkins
10:50–11:05 Intermission—Refreshments
11:05–11:25 Adrenals (Cortex and Medulla) and Hypertension—Dr. Bartels
11:25–12:00 Case Presentations
12:00–12:30 Questions and Answers—Dr. Wilkins, Moderator
12:30– 2:00 Luncheon
2:00– 2:05 Physiology of Testis—Dr. Heller
2:05– 2:20 Seminiferous Tubule Failure (Klinefelter's Syndrome)—Dr. Reifenstein
2:20– 2:50 Functional Castrates—Hypogonadotropic Eunuchoidism—Hypogonadism Secondary to Pituitary Failure—Dr. McCullagh
2:50– 3:10 Male Climacteric and Uses of Androgens in the Male—Dr. Heller
3:10– 3:25 Intermission—Refreshments
3:25– 3:35 Gynecomastia—Dr. Turner
3:35– 3:50 Tumors of Testis—Dr. McDonald
3:50– 4:15 Case Presentations
4:15– 4:45 Questions and Answers—Dr. Lisser, Moderator
4:45– 5:15 Ovarian Physiology—Dr. Albert

Wednesday, July 4, 1951

OVARIES, PREGNANCY

9:00– 9:30 Diagnostic Tests of Gonadal Function—Dr. Albert
9:30– 9:50 Metropathia Hemorrhagica—Dr. Allen
9:50–10:10 Dysmenorrhea and Endometriosis—Dr. Allen
10:10–10:20 Female Hypogonadism, Ovarian Agenesis and Turner's Syndrome—Dr. Turner
10:20–10:35 Premenstrual Tension—Mastodynia—Dr. Allen
10:35–10:50 Intermission—Refreshments
10:50–11:05 Amenorrhea—Dr. Reifenstein
11:05–11:25 Menopause—Dr. Rogers
11:25–11:45 Chronic Cystic Mastitis, Micro and Macromastia, Lactation—Dr. Allen
11:45–12:30 Variations in the Patterns of Adolescence; Precocious and Delayed Puberty—Dr. Wilkins
12:30–12:45 Chorionepithelioma and Hydatidiform Mole—Dr. deAlvarez
12:45– 2:00 Luncheon
2:00– 2:30 Male and Female Sterility—Dr. Heller
2:30– 3:00 Habitual Abortion, Toxemias of Pregnancy, Pregnancy in Diabetics—Dr. Allen
3:00– 3:20 Hormonal Treatment of Cancer of Breast and Prostate—Dr. McDonald
3:20– 3:40 Ovarian Tumors—Dr. Allen
3:40– 3:55 Intermission—Refreshments
3:55– 4:30 Case Presentations
4:30– 5:00 Questions and Answers—Dr. Turner, Moderator

Thursday, July 5, 1951

PITUITARY, PARATHYROID, BONE, NUTRITION, PSEUDO-ENDOCRINOPATHIES, NEUROENDOCRINES

9:00– 9:20 Neurohypophyseal Interrelationships—Dr. Patton
9:20– 9:40 Psychodynamic Aspects of Endocrinology—Dr. Shelton

9:40– 9:55	Tumors of Pituitary—Dr. McCullagh
9:55–10:05	Treatment of Acromegaly—Dr. Lisser
10:05–10:25	Simmonds' Disease and Anorexia Nervosa—Dr. Lisser
10:25–10:35	Diabetes Insipidus—Dr. Vidgoff
10:35–10:55	Hypo- and Hyperparathyroidism—Dr. Reifenstein
10:55–11:10	Intermission—Refreshments
11:10–12:05	Osteomalacia, Osteoporosis and Other Metabolic Bone Diseases—Dr. Reifenstein
12:05–12:30	Hormones and Nutrition—Dr. Albert
12:30– 2:00	Luncheon
2:00– 2:40	Endocrine and Other Factors Concerned with Somatic Growth—Dr. Wilkins
2:40– 2:50	Androgen Therapy of Prematurity—Dr. Shelton
2:50– 3:10	Hypothyroidism in Children—Dr. Wilkins
3:10– 3:25	Intermission—Refreshments
3:25– 3:45	Obesity and Thinness—Dr. Williams
3:45– 4:10	Hormone Preparations; Abuse of Hormone Therapy—Dr. Shelton
4:10– 5:00	Questions and Answers—Dr. Shelton, Moderator

Friday, July 6, 1951

Dr. Lester Palmer: Presiding—Opening Remarks

8:30– 9:15	Modern Concepts and Recent Experimental Aspects of Diabetes—Dr. Henry Ricketts
9:15– 9:45	Physiological Action of Insulin—Dr. Peter Forsham
9:45–10:15	Diagnostic Problems and the Proper Use of Laboratory Tests—Dr. Francis Lukens
10:15–10:30	Intermission and Viewing of Exhibits
10:30–11:30	Clinic: Presentation of Cases: Discussants—Drs. Blair Holcomb, Perry McCullagh, Henry Rickets, Howard Root, and Peter Forsham
11:30–12:00	Selection of the Proper Diabetic Diet—Dr. Henry Ricketts
12:00–12:30	Demonstration of Teaching of a Class of Diabetics by the Physician and Dietitian—Dr. Blair Holcomb
12:30– 2:00	Luncheon

Dr. Howard Root: Presiding

2:00– 2:30	Various Insulins and How to Use Them—Dr. James Sherrill
2:30– 3:00	Insulin Problems: Sensitivity, Resistance, Allergy, Local Lipodystrophy—Dr. Francis Lukens
3:00– 3:45	What Constitutes Diabetic Control—Long Term Results—Dr. Howard Root
3:45– 4:00	Intermission and Viewing of Exhibits
4:00– 4:45	Diabetes in the Surgical Patient—Dr. Perry McCullagh
4:45– 5:45	Panel: Questions and Answers
8:00	Public Meeting—Panel from the Faculty for the Seattle Diabetes Lay Society, Medical School Auditorium, University of Washington

Saturday, July 7, 1951

Dr. James Sherrill: Presiding

8:30– 9:30	Presentation of Cases: Discussants—Drs. J. Sherrill, L. Palmer, H. Rush and F. Lukens

9:30–10:00	Diabetic Pregnancy—Dr. Lester Palmer
10:00 10:30	The Diabetic Child—Dr. James Sherrill
10:30–10:45	Intermission and Viewing of Exhibits
10:45–11:15	Diabetic Retinopathy and Nephropathy, and Other Renal Complications—Dr. K. Swan
11:15–11:45	Diabetic Neuropathy—Dr. H. Ricketts
11:45–12:30	Diabetic Statistics and How To Use Them—Dr. H. Root
12:30– 2:00	Luncheon

Dr. Francis Lukens: Presiding

2:00– 2:30	Disorders of the Heart and Large Blood Vessels in Diabetics—Dr. Homer Rush
2:30– 3:00	Peripheral Vascular Disease and Gangrene—Dr. K. Swan
3:00– 3:45	Treatment of Diabetic Coma—Dr. H. Root
3:45– 4:00	Intermission and Viewing of Exhibits
4:00– 4:30	The Future of Diabetes and Its Prevention—Dr. J. Sherrill
4:30– 5:30	Panel: Questions and Answers

ENDOCRINOLOGY INDEX

The Association for the Study of Internal Secretions has undertaken to prepare a comprehensive Index of the articles published in *Endocrinology*. This Index will appear in three volumes. Volume III, covering papers published in 1940 through June, 1947, in volumes 26–40 of the journal, has been completed and is now available for distribution. Volumes I and II of the Index have not yet been completed.

Volume III of the Index is available gratis to members of the Association for the Study of Internal Secretions. The price to all non-members is $2.50. Inquiries and orders should be addressed to:

Dr. H. H. Turner, Secretary
1200 North Walker Street
Oklahoma City 3, Oklahoma.

BOOKS RECEIVED

THE following list acknowledges receipt of books which it has not yet been possible for us to review:

A Classified Bibliography of Gerontology and Geriatrics. By NATHAN W. SHOCK, Chief, Section on Gerontology, National Heart Institute, National Institutes of Health, Bethesda; and The Baltimore City Hospitals, Maryland. Prepared for Stanford University, under a grant from The Forest Park Foundation, Peoria, Illinois; 626 pages, 1951, Stanford University Press, Stanford, California. Price $15.00.

La stérilité involontaire. Évaluation des méthodes de diagnostic et de traitement. By RAOUL PALMER (Chef des Travaux de Gynécologie à la Faculté de Paris) with the collaboration of ÉLISABETH PALMER; 494 pages; 88 figures; 1950. Masson et Cie, Paris.

Le distrofie di origine diencefalo-ipofisaria. By Prof. C. GAMNA and F. CERESA; 277 pages, 1950. Ditta Luigi Pozzi, Editore, Roma.

Patterns of Sexual Behavior. By CLELLAN S. FORD, Associate Prof. of Anthropology, Yale University, and FRANK A. BEACH, Prof. of Psychology, Yale University, New Haven; 314 pages; 8 tables; 16 figures; 1951. Harper & Brothers, and Paul B. Hoeber Inc., New York. Price $4.50.

Pharmacological Basis of Penicillin Therapy. By KARL H. BEYER, Director of Pharmacological Research, Sharp & Dohme, Inc., Glenolden, Pa. A monograph in American Lectures in Pharmacology, edited by CHAUNCEY D. LEAKE; 230 pages; 17 tables; 35 figures; 1950. Charles C Thomas, Publisher Springfield, Ill. Price $4.50

Stress. By HANS SELYE, Professor and Director of the Institut de Médecine et de Chirurgie expérimentales, Université de Montréal. A treatise based on the concepts of the general adaptation syndrome and the diseases of adaptation; 842 pages, with an additional 203 pages of references; several illustrations; 1950. Acta Inc., Medical Publishers, Montreal, Canada.

Transactions of the American Goiter Association for 1950. Twenty-nine articles, with discussions, by leading authorities on the subject of the thyroid gland; 462 pages, with numerous tables and illustrations; 1951. Charles C Thomas, Publisher, Springfield, Illinois. Price $11.50.

Trends in Gerontology. By NATHAN W. SHOCK, Chief, Section on Gerontology, National Heart Institute, National Institutes of Health, Bethesda, and The Baltimore City Hospitals, Maryland; 162 pages; 9 tables; 12 figures; 1951. Stanford University Press, Stanford, California. Price $2.50.

Urologische Endokrinologie. By RUDOLF CHWALLA, Vienna; 560 pages; 18 illustrations; extensive bibliography; 1951. Springer-Verlag, Vienna. Price $13.—, geb. $13.80.

Vitamin E: Annotated Bibliography, 1940 to 1950. By P. L. HARRIS and W. K. KUJAWSKI; 184 pages. The National Vitamin Foundation, Inc., New York, N. Y.